STUDIES IN FOREST PATHOLOGY

II. THE BIOLOGY OF *FOMES PINICOLA* (SW.) COOKE

By
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TABLE OF CONTENTS*

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STUDIES IN FOREST PATHOLOGY

II. THE BIOLOGY OF *FOMES PINICOLA* (SW.) COOKE*

BY

IRENE MOUNCE, *Assistant Plant Pathologist*

I. INTRODUCTION

It was not until Robert Hartig (25) published the results of his studies of diseased wood and of his experiments with artificial infection that the disease and decay caused by wood-destroying fungi were actually attributed to them. Hartig's discoveries aroused a deep interest in such questions as the distinguishing characters of various rots, the hosts which are most susceptible, the prevalence of certain diseases in certain areas and the conditions which favour their development, prophylactic measures, and, based upon all of these, the formulation of plans for the most economic harvesting of timber.

As these studies of wood-destroying fungi progressed, the need of correlating the data collected in the field with results of detailed laboratory experiments became increasingly evident. Some rots macroscopically indistinguishable are due to different organisms; some rots vary in appearance on different sub-strata and under varying conditions of growth; and, in the absence of sporophores, the identity of the causal organisms in such cases can be determined accurately only by cultures. Again, no specific method of adequately dealing with a disease can be devised until the mode of dissemination and infection, in fact, until the whole life history of the organism is known.

Until recently this phase of forest pathology had received comparatively little attention. However, the work of Long and Harsch (34), of Fritz (21), and of Hubert (28) has supplied criteria for the identification of many wood-destroying fungi from their cultural characters; and the work of Buller (10), Bayliss (3), Rhoads (51), Hiley (27), White (76), Zeller (77), Kauffman (29), and others has supplied us with details of the life histories of such wood-destroying fungi as *Polyporus squamosus, Lentinus lepideus, Polystictus versicolor, Polyporus pargamenus, Lenzites saepiaria, Dasyscypha calycina, Fomes annosus, Polyporus dryophilus, Fomes applanatus,* and *Trametes robiniophila.* It is with this type of investigation, in its broader aspects, that this paper is largely concerned. The subject chosen is *Fomes pinicola.*

Fomes pinicola is one of the very common wood-destroying fungi in both Europe and America. Since it produces large, conspicuously coloured sporophores (plate I) and destroys both sapwood and heartwood of coniferous and deciduous trees, reports of its occurrence and distribution are frequent throughout the literature dealing with the field aspects of forest pathology. In addition, observations on the cultural characters of this fungus have been made by Rumbold (55), Long and Harsch (34), Fritz (21), and Schmitz (57); but beyond this it has received practically no attention. The present paper summarizes the results of those laboratory studies, and then goes on to include such phases as spore germination; the mycelial characters of the fungus when grown on liquid and solid media; temperature as a factor influencing mycelial development; sporophore production on artificial media and on wood blocks; a comparative study of monosporous and polysporous mycelia; sexuality; and macroscopic and microscopic features of the decay caused by *F. pinicola.*

* A thesis submitted in conformity with the requirements for the degree of Doctor of Philosophy in the University of Toronto.

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II. HISTORICAL

Because of the frequency of its occurrence on so many different hosts and in so many localities, *F. pinicola* not only appeared early in mycological literature but also under a variety of names. Murrill (40) gives the following list of synonyms:—

Boletus igniarius Scop. Fl. Carn. ed. 2.2:469. 1772. Not *B. igniarius* L. 1753.
Boletus ungulatus Schaeff. Fung. Bavar. 4:88, *pl. 137. 138.* 1774.
Boletus fulvus Schaeff. Fung. Bavar. 5:89, *pl. 262.* 1774.
Boletus semiovatus Schaeff. Fung. Bavar. 4:92. *pl. 270.* 1774.
Boletus marginatus Pers. Obs. Myc. 2:6. 1799.
Boletus pinicola Sw. Sv. Vet. Akad. Handl. 1819:88. 1810.
Polyporus marginatus Fries. Syst. Myc. 1:372. 1821.
Polyporus pinicola Fries. Syst. Myc. 1:372. 1821.
Fomes marginatus Gill. Champ. Fr. 1:683. 1878.
Fomitopsis pinicola Karst. Rev. Myc. 3^9:18. 1881.
Fomes pinicola Cooke. Grevillea 14:17. 1885.
Fomes ponderosus Schrenk. Bull. U.S. Dep. Agr. Pl. Ind. 36:30. 1903.
(Type from Dakota, on *Pinus ponderosa.*)

Fries (20) considered the variations in form, colour, and zonation of the sporophores of this fungus on coniferous and deciduous hosts distinct enough to be specific and he has two species, *P. pinicola* and *P. marginatus,* based on such differences. Saccardo (56) recognises Fries' species and creates a third *F. ungulatus.*

He gives the following synonyms for each:

Fomes pinicola Fr. Elench. p. 105. Hym. Eur. p. 561. Gill. Champ. c. ic.
Agaricus sitaneus, subrotundus, fulvus, margine obtuso, pallido, stillante.
Boletus fulvus Schaeff. t. 262.
B. marginatus Pers. Syn. p. 534. Swartz. Vet. Akad. Handl. 1810. p. 87.
B. hornotinus et *annosus,* pileo ungulato, nigricante, margine cinnabarino.
B. semiovatus Schaeff. t. 270.(varietas).
B. igniarius Fl. Dan. t. 953. Pers. Syn. p. 534.
B. pinicola Swartz. l.c. p. 88. Wahlenb. Suec. n. 2009.
B. marginatus Weinm.

Fomes ungulatus (Schaeff.) Sacc. Michelia I. p. 539.
Boletus ungulatus Schaeff. Fung. Bat. t. 137 (ab auctoribus non citata), nec Bull.

Fomes marginatus Fr. Epicr. p. 468. Hym. Eur. p. 561. Quélet. t. 19, f. 2 (var.)

Saccardo states that his *F. ungulatus* may be only a form of *F. pinicola.* Later workers, however, including Hedgecock (26), Lloyd (33), Murrill (40), and Overholts (47) consider the three species given by Saccardo as forms of one species which they call either *F. pinicola* or *F. ungulatus,* depending on whether or not they accept Fries' *Systema mycologicum* as the starting point of mycological nomenclature. A study of the sporophores of this fungus, and of the behaviour of monosporus mycelia in paired cultures (section XIII) has

led the author to the same conclusion, and throughout this paper *Fomes ungulatus* (Schaeff.) Sacc. and *F. marginatus* have been considered as forms of *F. pinicola* (Sw.) Cooke.

While collecting on the Queen Charlotte Islands, B.C., where western hemlocks are numerous and *Fomes pinicola* particularly abundant, groups of sporophores were examined to see whether the majority more closely resembled the applanate form with soft context and pale margin, characteristic of *P. marginatus* Fr., or the ungulate form with vermilion red margin and hard context characteristic of *P. pinicola* Fr. Since all of the sporophores were collected from a coniferous host the great majority would be expected to resemble the *P. pinicola* form which was supposedly the common one on coniferous hosts, rather than the *P. marginatus* form which was thought to grow chiefly on deciduous hosts. As a matter of fact both types of sporophores and all gradations between the two were common. Some sporophores had a soft context, some a hard, some had broad, white margins, some vermilion red margins, with many showing intermediate buff-coloured tints. The sporophores were applanate or ungulate in shape, the upper surface rough and zoned, or smooth, completely covered with a shining red lacquer, or entirely dull grey, brown, or black, with all the intermediate forms with vari-coloured margins and vari-coloured zones. Groups of sporophores showing both type forms and many intermediate ones could be collected from one tree.

Cultures were made from the inner tissues of a large number of sporophores, but it has not been found possible to associate any specific variations in cultural characters with specific differences in sporophore form. Furthermore, monosporous mycelia isolated from cultures 5778, 928, and 586 are all mutually fertile when paired with monosporous mycelia isolated from cultures 283, 285A, 285B, and 5770 (See section XIII). In this *F. pinicola* behaves similarly to other species of Basidiomycetes which have been investigated by Kniep (32), Vandendries (68-9), Brunswik (9), and Hanna (24). But culture 5778 was made from a sporophore which grew on *Prunus serotina,* culture 928 was from *Betula* sp. and collected as typical of the *F. marginatus* form of Europe, and culture 586 was labelled *F. pinicola* var. *marginatus.* The remaining cultures were isolated from sporophores which grew on *Picea* and would formerly, in all probability, have been identified as *F. pinicola.* The fact that monosporous mycelia from 5778, 928, and 586 are all mutually fertile with those from cultures 283, 285A, 285B, and 5770 is taken as contributory evidence that all of the sporophores, from which these cultures were made, belong to one and the same species. This conclusion is based upon the clamp-connection criterion for the identity of species which Vandendries (68) states as follows, " Si les haplontes de deux carpophores sauvages sont toujours et indéfiniment fertiles entre eux ces deux carpophores appartiennent à une même espèce."

III. DISTRIBUTION

A. GEOGRAPHICAL

Fomes pinicola occurs frequently both in Europe and America. Its presence in exsiccati from France, Scandinavia, Saxony, Bavaria, Austria, Hungary, and Italy, indicates its distribution in Europe. It is reported from Uruguay, Cuba, and Asiatic Siberia, and Lloyd (33) has specimens, as well, from Japan, the Philippine Islands, and Mexico. Of its distribution in America Hedgcock (26) says, " it is one of the most widely distributed species in the United States occurring, as far as has been observed, in every region where conifers are found," and this is corroborated by the reports of Meinecke (35), Weir (72), Rankin

(50,), and others. The same might be said of its distribution in Canada, for the fungus is common in the forests of British Columbia, and is found from Manitoba to the Atlantic. Weir (74) has collected it in the mountains at altitudes of 6,735, 7,600, and 8,500 feet.

B. HOST LIST

Although *Fomes pinicola* is most commmonly found on coniferous trees it occurs on deciduous ones as well. The host list which follows includes 91 species and has been compiled from the reports or collections of Atkinson (1), Boyce (6), Cutler (12), Dudley (13), Farlow and Seymour (16), Graves (22), Faull (17, 18), Hedgcock (26), Long and Harsch (34), Neuman (41), Oudemans (46), Pennington (48), Saccardo (56), von Schrenk (58, 59, 60), Sydow (67), Spaulding (65), Weir (72), and Zeller (75). The list contains all the hosts which have been found recorded for *F. pinicola* but does not attempt to include every locality from which it has been reported on each host nor the names of every collector who has reported it.

CONIFEROUS HOSTS

Host	Locality	Collected or reported by
1. *Abies alba*		Oudemans
2. " *albicaulis*	United States	Weir, Hedgcock
3. " *amabilis*	"	"
4. " *aristata*	"	"
5. " *arizonica*	"	"
6. " *attenuata*	"	"
7. " *balsamea*	Canada, Ontario	Faull
" "	United States, Michigan	Pennington
8. " *cembroides*	" "	Hedgcock
9. " *chihuahuana*	" "	"
10. " *concolor*	" "	"
11. " *contorta*	"	Hedgcock, Weir
12. " *divaricata*	"	"
13. " *echinata*	"	"
14. " *edulis*	"	"
15. " *excelsa* (*Picea*)	Germany	Sydow
16. " *flexilis*	United States	Hedgcock, Weir
17. " *grandis*	"	" "
18. " *lasiocarpa*	"	" "
19. " *magnifica*	"	"
20. " *nobilis*	"	"
21. " *shastensis*	"	"
22. *Larix americana*		Farlow and Seymour
" "	United States, New York	Dudley
23. " *europaea*	Europe	Saccardo
24. " *laricina*	United States	Hedgcock
" "	" Wisconsin	Neuman
" "	Canada, Ontario	Faull
25. " *Lyallii*	United States	Hedgcock, Weir
26. " *occidentalis*	"	"
" "	" Idaho	Long and Harsch
27. *Picea canadensis*	Canada, Ontario	Faull
" "	United States	Hedgcock
28. " *Engelmanni*	"	" Weir
29. " *excelsa*		Oudemans
30. " *mariana*	United States	Hedgcock, Weir
" "	" Wisconsin	Neuman
" "	Canada, Ontario	Faull
31. " *Parryana*	United States	Hedgcock
32. " *rubens*	"	"
33. " *sitchensis*	Canada, British Columbia	Cutler
" "	United States	Hubert
34. *Pinus Banksiana*	Canada, Ontario	Mounce
35. " *glabra*	United States	Hedgcock
36. " *Jeffreyi*	"	"

CONIFEROUS HOSTS—Concluded

Host	Locality	Collected or reported by
37. *Pinus Lambertiana*	Uuited States	Hedgcock
38. " *Mayriana*	"	"
39. " *monophylla*	"	"
40. " *monticola*	"	" Weir
41. " *palustris*	"	"
42. " *pinaster*		Oudemans
43. " *ponderosa*	United States	Hedgcock, Weir
" "	" Dakota	von Schrenk
44. " *resinosa*	"	Hedgcock
" "	Canada, Ontario	Faull
45. " *rigida*	United States	Hedgcock
46. *Sabiniana*	"	"
47. " *strobiformis*	"	"
48. " *Strobus*	"	"
" "	" Wisconsin	Neuman
" "	Canada, Ontario	Faull
49. " *sylvestris*	Europe & Asiatic Siberia	Saccardo
50. " *taeda*	United States	Hedgcock
51. " *virginiana*	"	"
52. *Pseudotsuga taxifolia*	" Montana	" Weir
" "	" New Mexico	Long and Harsch
" "	" Washington	Bartholomew
" "	Canada, British Columbia	Cutler
53. *Tsuga canadensis*	United States, Wisconsin	Neuman
"	" New York	Dudley
"	" North Carolina	Graves
"	Canada, Ontario	Faull
54. " *heterophylla*	United States	Hedgcock
" "	" Montana	Weir
" "	Canada, British Columbia	Cutler
55. " *Mertensiana*	Canada, British Columbia	Cutler
" "	United States	Hedgcock

DECIDUOUS HOSTS

56. *Acer saccharinum*	United States	Hedgcock
57. " *saccharum*	" Adirondacks	Atkinson, Pennington
" "	" "	Spaulding
58. *Alnus glutinosa*	Germany, Baden	Weir
59. " *incana*	Canada	Faull
60. " *oregona*	United States	Hedgcock
61. " *tenuifolia*	"	"
" "	" Montana	Weir
62. *Betula spp*	Europe	Saccardo
63. " *fontinalis*	United States	Hedgcock
64. " *lenta*	"	" Spaulding
" "	" Adirondacks	Atkinson, Pennington
65. " *lutea*	"	Hedgcock
66. " *occidentalis*	"	"
" "	" Montana	Weir
67. " *papyrifera*	"	Hedgcock
" "	Canada, Ontario	Faull
" "	United States, Wisconsin	Neuman
68. " *verrucosa*		Oudemans
69. *Carya* sp	United States, Wisconsin	Neuman
70. *Castanea vesca*		Oudemans
71. *Fagus atropunicea*	United States	Hedgcock
72. " *ferruginea*	" Adirondacks	Atkinson, Pennington
" "	Canada, Quebec	Mounce
73. " *sylvatica*	Germany, Baden	Weir
" "		Saccardo
74. *Magnolia foetida*	United States	Hedgcock
75. *Platanus occidentalis*		Oudemans
76. " *orientalis*		"
77. *Populus balsamifera*	United States	Hedgcock
" "	" Michigan	Pennington
78. " *grandidentata*	"	Hedgcock
" "	N.E. United States	Spaulding
" "	Canada, Ontario	Faull
79. " *tremula*		Saccardo

Deciduous Hosts—Concluded

Host	Locality	Collected or reported by
80. *Popolus tremuloides*	United States	Hedgcock, Weir
" "	N.E. United States	Spaulding
81. " *trichocarpa*	United States	Hedgcock, Weir
82. *Prunus avium*		Oudemans
83. " *Cerasus*	United States	Zeller
84. " *persica*	"	Farlow and Seymour
85. " *serotina*	"	Hedgcock, Pennington
" "	"	Spaulding
" "	Canada, Ontario	Faull
" sp		Oudemans
86. *Pyrus communis*		Oudemans
87. " *Malus*	United States, Idaho	Weir
88. *Quercus pedunculata*	Germany, Baden	"
" spp		Saccardo
89. *Salix lasiandra*	Canada, Selkirk Mts	Weir
90. *Amygdalus persica*	United States, Oregon	Zeller
91. *Prunus domestica*	" "	Zeller

IV. OCCURRENCE

Fomes pinicola usually grows saprophytically, destroying wood in stumps and fallen logs, or in standing trees which have been killed through some other agency, such as fire, bark beetles, or budworm. Although there is evidence to show that it may grow and produce sporophores on living trees, so far, *no definite proof of its parasitism,* such as White (76) found for *F. applanatus* by the artificial infection of living trees and by showing, microscopically, that the hyphae attack living tissues, has been obtained.

As early as 1894 Nilsson (44, 45) reported *F. pinicola* attacking the spruce trees of the Norrland and Dalecarlian forests (Kopparberg). He described the gradual browning and cracking of the wood of infected trees, and considered this fungus responsible for their death. In writing of the diseases of conifers in the forests of Sweden the same author states that *F. pinicola* is parasitic in Southern Norrland and in Dalecarlia, and saprophytic in Southern Sweden.

References to the occurrence of *F. pinicola* on living trees in America are common. In 1901 Atkinson (1) reported, "The fruit-bodies of *Polyporus pinicola* are sometimes found on the trunks of living hemlocks where it is apparently a wound parasite, entering by means of old knot-holes, branch wounds, fire scars, and similar injuries." Hedgcock (26) finds it attacking " living trees to some extent gaining entrance through heartwood or sapwood as it rots both readily." Graves (22) reports *F. pinicola* as the cause of a rot of hemlock adding that it is " a dangerous enemy, not only because of its omnivorous habit, but chiefly on account of its ability to attack *living* as well as dead trees." Meinecke (35, 36) states that it is the commonest timber-destroying pore-fungus in California " exceedingly destructive to fallen timber, and may cause damage to *living* trees as well;" and, again, " The writer has found it on thrifty sugar pine in Central California, where it was undoubtedly parasitic in the sense of attacking the sound heartwood of living trees through an open fire scar, and extending toward the sapwood." Zeller (79) finds *F. pinicola* to be the cause of heartrot in the wood of peach (*Amygdalus persica*) and of prune (*Prunus domestica*) trees. Neuman (41) found sporophores of this fungus on *living* and dead trees of hemlock, tamarack, birch, white and red pine, and spruce. The same author gives a

detailed description of *F. pinicola* as a parasite on tamarack. There was a large wound at the base of the tree and, on this, sporophores of the fungus had developed. When the tree was cut down and examined, it was found that near the wound the rot had reduced the entire heartwood and most of the sapwood to a brittle brown condition, and it had spread upward to a height of eight feet from the base of the tree. In spite of this the top of the tree was still green, though it had a sickly appearance, and some of the lower branches were dead or losing their needles. Weir (72) cites a similar instance in which this fungus was found on a wound on a living apple tree. However, as Weir (75) explains later, "In the writer's opinion the term 'wound parasite' as it is ordinarly employed is misleading. The parasitism of but few wood-destroying fungi has been investigated. The mere fact that they are found growing from wounds does not imply that they would attack the living cell. *Fomes pinicola*, one of the most common saprophytes, chiefly on coniferous wood, not unfrequently enters through wounds and destroys the heartwood of living trees, but it would not be considered parasitic." Mr. A. W. McCallum collected, at Gaspé, Quebec, *F. pinicola* on living *Abies balsamea* where it caused a typical butt-rot. The fungus had probably entered through a root which was adjacent to infected stumps.* Boyce (6) in writing of decay in Sitka spruce (*Picea sitchensis*) says, "The red belt *Fomes* which is most common on snags, windfalls, and other debris, also causes a butt rot in living trees. . . . . The typical decay is light reddish brown in colour, crumbly and brittle, breaking up into rough cubes with mycelium felts in the cracks." Hubert, in a letter dated January 19, 1926, writes as follows, with reference to a brown rot of spruce, "I know that *Fomes pinicola* and *Polyporus Schweinitzii*, both producing brown rots, are common in living Sitka spruce." The writer has seen a sporophore of *F. pinicola* on a living Douglas fir (*Pseudotsuga taxifolia*), and, during the summer of 1925, collected specimens of typical *Fomes pinicola* rot from two living trees of Sitka spruce at Queen Charlotte City, B.C. Cultures were made from both specimens, and mycelium typical of *Fomes pinicola* obtained. So far no sporophores have developed in culture, so that identification is not absolute, but there seems little doubt that these results are in accordance with Dr. Hubert's statement. Rankin (50) seems to sum up the opinions cited above when he states that *F. pinicola* "occurs less frequently in living trees than it does on dead standing trees and logs," but that it may attack trees which have been wounded, or are in generally poor health.

In any case *F. pinicola* is everywhere the cause of serious loss through its rapid destruction of dead standing timber. Von Schrenk (60) describes conditions in the Black Hills Forest Reserve, where stands of Western Yellow Pine trees which had been killed by beetles were soon worthless through the activity of this fungus. He found a similar condition in an area which had been burned over four years previously (58). His field observations led him to conclude that, "as a result of the growth of this fungus, where the timber on forest lands has been killed it will be found practically valueless after six or eight years at the most." Von Schrenk's conclusions are substantiated by the observations of Humphrey (53) and others made in Eastern Canada where areas of spruce and balsam had been killed by the spruce bud-worm. Experiments have been made on the utilization of wood infected with *F. pinicola*, for pulp (54). This fungus belongs to the group which destroys cellulose, and as a result, the yields from both early and advanced stages of rot are very low, and the pulp itself is of little value.

*Report of the Dominion Botanist, Department of Agriculture, Ottawa, for the year 1927, page 40.

V. THE SPOROPHORE

The rapidity with which *Fomes pinicola* spreads over large areas of dead standing timber is due, in part at least, to its prolific production of large sporophores (plate I), which live for many years and increase their pored surface each year. It is not uncommon to find twenty-five or more of these large brackets on one side of the trunk of a western hemlock, and on Queen Charlotte Islands 121 sporophores were counted on a hemlock trunk (*Tsuga heterophylla*), of which only about 40 feet were left standing. The spores are carried in all directions by the wind and sooner or later find any exposed surfaces on the neighbouring trees. Beetle holes afford an easy means of entrance and Spaulding (64) reports that the mycelium of *F. pinicola* has been found frequently in them. Von Schrenk (58) makes the further suggestion that the holes made by wood-boring insects "may serve to account for the fact that the decay of a large tree takes place with such rapidity."

The sporophores of *F. pinicola* vary a great deal in form and colour. Murrill (40) describes them as ungulate, Overholts (47) as convex or ungulate. They are as often broadly applanate (plate I, fig. 2) as they are ungulate (plate I, fig. 1) even in the same locality and on similar hosts. The upper surface in some specimens is definitely zoned; in others only sulcate with age. There is as wide a variation in colour as there is in form. Young sporophores may have the upper surface entirely covered with a clear, resinous, sticky, varnish-like coating ranging in colour from ferruginous and cinnamon-rufous to Hays russet and Kaiser brown (Ridgway, 52); or the lower edge of the sporophore may consist of a broad band, even 5 cm. wide, which is identical in colour with the hymenium and in marked contrast to the reddish brown varnished appearance of the upper surface (plate I, fig. 2). In older sporophores the upper surface becomes hard, roughened, and dark in colour, more or less mottled with smoke grey, greyish olive, seal brown, and black, but usually retaining at the margin a band of varying width which exhibits the reddish brown tones found on the young sporophore. These sporophores eventually become very large. Overholts (47) gives the fruit-body measurements as 4-15 by 6-20 cm.; Dr. Faull collected a beautiful specimen at Timagami, Ontario, which measured 20 by 36 cm.; while Schulz (61) found two specimens, on dead stumps near Vigny, which measured 39 by 20 cm. and 45 by 24 cm. respectively. Bataille (2) makes a note of the fact that ammonia causes the hymenium of *Polyporus marginatus* to become a rose red colour. A similar reaction is obtained when sporophores formed in cultures of *F. pinicola* are exposed to ammonia fumes.

In contrast to the variations in the colour of the exterior and in the form of the sporophore the colour and texture of the fruit-body context, the width and colour of the hymenial tubes, and the size of the spores remain fairly constant and offer reliable criteria for the identification of this fungus. The context is corky to woody in texture and more or less concentrically zoned; it ranges in colour from colonial buff and light ochraceous salmon to shades of pinkish and cinnamon buff; and in younger specimens it may reach a width of 5 cm. or more, while in the later growth of older specimens it gradually decreases until it is only a few millimeters in width. There may be, particularly in younger specimens, a layer of context ·5-3 mm. wide between the layers of tubes but this is not a constant character. The tubes are concolorous with the context and vary in colour as it does, except that the last formed hymenial layer may be lighter in colour than the others. The hymenium ranges in colour from white, light and pinkish buff, and light pinkish cinnamon, to shades of pinkish cinnamon. Around the pored surface, and concolorous with it, there is a sterile band from 2-5 mm. wide. The pores are small, 4-5 to a millimeter. The spores range in size

from 3.4 by 5 μ to 4.5 by 7.3 μ averaging 3.5 by 6.5 μ. They are ovate to subglobose, hyaline, thin-walled, with coarsely granular contents and occasionally oil drops (plate II, fig. 1).

Complete data regarding the spore discharge period of *F. pinicola* are not available. From the vicinity of Guelph, Ontario, Stone (66) reports a visible spore discharge from a sporophore of this fungus in May. The writer has obtained spore casts in the middle of May from sporophores collected by Dr. Faull at Timagami, Ontario, and in September from sporophores on western hemlocks in Stanley Park, Vancouver, B.C. Mr. G. D. Darker of the Department of Botany, University of Toronto, very kindly set spore-traps on Bear Island, Timagami, and kept records of spore fall from June-August, 1924. He reported the spore discharge continuous throughout June, no evidence of it during July, and a few spores caught in August. This would suggest that *F. pinicola,* like *F. applanatus,* has a spore discharge period of several months, but confirmation is necessary.

VI. PREVIOUS CULTURAL STUDIES

Cultural studies of *Fomes pinicola* have been made by Brefeld, Rumbold, Long and Harsch, Fritz, and Schmitz. Rumbold was interested particularly in the effects of the acidity of media upon mycelial development; Long and Harsch and Fritz in the cultural characters of the mycelium, and Schmitz in physiological specialisation as exhibited by this fungus.

Brefeld (7) tells us no more than that he received his material from Norway and that the spores of the fungus germinated rapidly, developing a large amount of aerial mycelium which in old cultures produced clamp-connections but remained sterile.

Rumbold (55) obtained her cultures from sporophore tissue. She describes in a general way the colour and texture of the mycelial mat and notes and figures the fine hyphæ with their clamp-connections. She grew the fungus on a series of tubes of media whose acidity was so adjusted that when tested with litmus they gave red, red violet, violet, blue violet, or blue colour. Of these the first three were acid to phenolphthalein, the fourth slightly alkaline, and the fifth alkaline. When the mycelium of *F. pinicola* was grown on this series it was found that the greatest amount of growth occurred on the most acid medium and that the amount of growth decreased with the acidity, until only a slight growth occurred on the neutral medium and none at all on the alkaline one.

Long and Harsch (34), too, used mycelium from sporophore tissue in their study of *Fomes pinicola.* One strain was obtained from a sporophore which grew on *Pseudotsuga taxifolia,* the other from a sporophore which grew on *Larix occidentalis.* No detailed results of their studies are given in their preliminary paper except that both strains of the fungus produced a white mycelium and sporophores. The strain from *Pseudotsuga* produced a sporophore in malt cultures both in the presence and absence of light, and after a period of 77 days. The strain from *Larix* produced sporophores only in the light, and on parsnip and prune agars. The sporophore on the prune agar culture required 43 days to develop.

Fritz (21) includes *Fomes pinicola* among the seventeen wood-destroying fungi for which she worked out a system of identification by cultural characters. She studied and has described (1) the macroscopic features of the mycelial growth, such as the texture, colour, rate of growth, and manner of advance on the agar surface; (2) the microscopic features of the aerial and submerged mycelium

including the types and colour of the hyphæ, the method and frequency of branching, septation, occurrence and type of clamp-connections; and (3) the variations in the character of the mycelium which occur when the fungus is grown on potato dextrose, potato, malt, corn, and Czapek's and Currie's synthetic agars. The cultures for this work were obtained from sporophores and wood from dead *Populus grandidentata, Betula alba,* and *Abies balsamea,* from sporophores from dead *Pinus Strobus, Picea mariana, Larix americana, Tsuga canadensis,* and *Prunus serotina,* and from living *Abies balsamea.*

Schmitz (57) studied four strains of *Fomes pinicola,* which he obtained from sporophores from dead Douglas fir, white fir, western hemlock, and western white pine, with reference to physiological differences in (1) growth characters, (2) intracellular and extracellular enzyme activity, (3) the inhibiting effect of these strains upon one another, (4) growth upon media containing various substances as a source of nitrogen, (5) growth on liquid media, and (6) wood-destroying properties.

The four strains of *Fomes pinicola* were grown in large flasks on carrot. Under these conditions the strain from white fir gave a much heavier and more coriaceous mycelial mat than any of the others, while that from Douglas fir remained fluffy in appearance. The mycelial mats were removed from the flasks, dried and ground, and used in the study of intracellular enzymes, while the liquid in the flasks and juices extracted from the carrots were used in studying extracellular enzyme activity of the various strains.

This study showed that there was a positive and similar action for all strains in carbohydrase and catalase activity; negative results for all strains for urease and amidase; no definite results for rennetase and tannase activity; while slight differences occurred in the relative esterase and glucosidase activity.

Similarly a study of intracellular enzyme activity showed positive action and similar results for all strains in carbohydrase and tannase activity, negative in all for protease, and differences in esterase and glucosidase activity.

In all mixed cultures the effect of the various strains upon each other was evident. In all plates having two inoculations of the same strain there seemed to be no inhibiting or stimulating effect, the colonies grew into each other and covered the agar surface as if the growth had resulted from a single inoculation. On the other hand whenever plates were inoculated with two different strains the colonies did not intermingle. In these cases a clear line of demarcation remained between the two colonies (cf. plates VI and VII).

In order to study their wood-destroying properties the four strains of *F. pinicola* were grown for six months on weighed blocks of the following woods: heart and sapwood of *Pinus ponderosa,* heartwood of *P. monticola, Larix occidentalis, Pseudotsuga taxifolia, Abies grandis, Tsuga heterophylla,* and *Picea Engelmanni.* At the end of that time the blocks were dried and weighed and the loss of weight calculated. In only one case did the fungus in question cause a more rapid decay on the wood of the host from which it was originally obtained. The *white pine* strain caused the highest grand average of decay.

Schmitz summarises his results by saying, "The writer believes that the data presented are sufficiently conclusive to indicate that there may be considerable physiological variation within the species *Fomes pinicola* Fr. Whether or not this variation is the result of host influence is not certain."

VII. CULTURAL METHODS AND CONDITIONS OF GROWTH

A. ORIGINAL CULTURES

Cultures of the mycelium of *Fomes pinicola* were obtained from spores, from sporophore tissue, or from wood which had been decayed by *F. pinicola*. Small pieces from the interior of a sporophore or from a freshly exposed surface of the diseased wood were removed with sterile forceps and partially embedded in agar slants. Subcultures were made by removing a small piece of mycelium-covered agar with a sterile loop and placing it on a fresh agar slant.

Cultures were made on liquid and solidified media; those on liquid media were set up in 200 cc. Erlenmeyer flasks; those on solidified media were set up in test tubes 15 by 2·2 cm.; except that in one experiment Erlenmeyer flasks were used and, in another, glass culture tubes 2 by 12 inches.

B. CONDITIONS OF GROWTH

In most cases tube cultures were grown at room temperature and in diffuse light. The tubes were placed on a table in trays which were slanted at an angle of about 15° so that they received light from a window opposite them. For special experiments cultures were grown in incubators at 15°, 22°, 27°, 30°, and 35° C.

C. MEDIA

1. *Prune Agar.*—120 g. dried prunes were soaked overnight in 400 cc. distilled water and then steamed for one hour. 25 g. agar were dissolved in 750 cc. distilled water by steaming in the autoclave for 30 minutes at 15 pounds pressure. 250 cc. of the prune extract were added then to the melted agar, the whole filtered through cotton, tubed, and sterilized in flowing steam for 15 minutes on each of three successive days.

2. *Potato Dextrose Agar.*—This medium was made according to a formula given to me by Dr. Fritz, viz., 400 g. sliced potatoes and 1 l. distilled water were steamed in an autoclave 30 minutes at 15 pounds pressure and strained. The potato decoction thus obtained was used to replace the unabsorbed water drained from 25 g. shredded agar, which had been soaked overnight in 1 l. distilled water. The agar was then melted by steaming in an autoclave 20 minutes, at 15 pounds pressure, and on removal 25 g. dextrose were added before tubing. To prevent prolonged decomposition of the sugar from the action of heat, the autoclave was heated to steaming before the tubes were placed in it for sterilization. They were then sterilized 10 min. at 15 pounds pressure.

3. *Carrot, Celery, Bean, Pea, Cornmeal, and Parsnip Agars* were made in the same way as (2) with the omission of dextrose and using the following amounts of vegetables per litre: carrot 490 g.; celery 600 g.; bean 400 g.; parsnip 490 g.; pea 400 g.; cornmeal 80 g.

4. *Beet Agar* was made as for medium (1) using 490 g. of vegetable per litre.

5. *Czapek's Synthetic Agar.*

0·5 g. magnesium sulphate.
1·0 g. mono-potassium phosphate.
0·5 g. potassium chloride.
0·01 g. ferrous sulphate.
2·0 g. sodium nitrate.
30·0 g. dextrose.
25·0 g. agar.
1 l. distilled water.

6. *Czapek's Synthetic Agar (modified*—C. W. Fritz).—In this medium the sodium nitrate of medium (5) was replaced by 20 g. peptone which was dissolved with the dextrose in 200 cc. of the water used and added just before tubing.

7. *Malt Agar.*—25 g. malt extract, 25 g. agar were dissolved in 1 l. distilled water by steaming in an autoclave, for 20 minutes at 15 pounds pressure. The medium was then filtered, tubed, and sterilized as for medium (2).

8. *Czapek's Liquid Synthetic Medium.*—Used as for medium (5) with the omission of the agar.

9. *Czapek's Liquid Synthetic Medium (modified).*

20·0 g. glycerol.
2·5. g. asparagin.
0·5. g. magnesium sulphate.
0·5. g. potassium chloride.
0·01 g. ferrous sulphate.
1 l. distilled water.

10. *Prune Decoction.*—120 g. dried prunes were soaked in 1 l. distilled water overnight then placed in flowing steam for 1 hour. The decoction was then filtered, put in flasks, and sterilized in flowing steam for 20 minutes on each of three succesive days.

11. *Wood-block Cultures.*—Blocks of wood approximately 1·5 by 8 cm. were cut and repeatedly boiled in water and cooled to drive out the air. When the blocks sank they were placed in test tubes 15 by 2·2 cm. upon a layer of water-soaked cotton 2·5 cm. deep (Plate V). The tubes were then plugged and sterilized for 30 minutes at 15 pounds pressure.

Two cultures were made in which blocks of wood 2 by 2 by 3 inches were used (Plate X). They were repeatedly boiled and cooled and then placed in two-quart sealers upon a two-inch layer of water-soaked cotton, plugged, and sterilized as the others.

D. ACIDITY

With the exception of medium (9) no adjustment of the natural acidity of the medium was made. In that, by the addition of monobasic or dibasic potassium phosphate, the H ion concentration was adjusted colorimetrically to a degree of pH 4·2, 4·8, 5·2, 5·8, and 6·2.

VIII. SPORE GERMINATION

The spores used for the following experiments were obtained both from sporophores in the field and from those obtained in pure cultures grown on prune agar. The spores were sown in the usual way in sterile van Tieghem cells and except in special experiments were incubated at a temperature of 22°-24° C.

A. SUBSTRATA USED

Spores of *Fomes pinicola* were sown in hanging drops of the following substrata: bean, carrot, cornmeal, beet, green pea, parsnip, potato dextrose, sweet potato, and malt agars; malt gelatine; pine wood decoction; 1 per cent solutions of maltose, dextrose, and lactose; 0·4 per cent solutions of sodium chloride, mercuric chloride, ammonium phosphate, monobasic potassium phosphate, dibasic potassium phosphate, copper sulphate, and sodium carbonate; tap water, distilled water, and distilled water containing traces of acetic acid and of alcohol. The spores failed to germinate in tap water, pine wood decoction, and solutions of acetic acid, alcohol, mercuric chloride, copper sulphate, sodium carbonate,

and dibasic potassium phosphate. They germinated in all the other substrata, though there was little further development in either the sodium chloride or ammonium phosphate solutions. The spores germinated most readily and produced the greatest mycelial development in the 1 per cent solution of lactose, and in potato dextrose, beet, parsnip, carrot, and bean agars.

B. ACIDITY

No definite study of the effect of variations in the acidity of the medium upon spore germination were made. However, since the spores of *F. pinicola* germinate in cornmeal, potato, carrot, prune, bean, and beet agars, and since Duggar, Severy, and Schmitz (14) found the acidity of those plant decoctions to be (Fuller Scale): cornmeal + 3; potato + 11·5; carrot + 13·5; prune + 14·5; bean + 15 and sugar beet + 22·6, it would seem that, at least within a wide range, acidity of medium is not a limiting factor.

C. TEMPERATURE

Spores of *Fomes pinicola* were found to germinate at temperatures ranging from 8°-35° C. and a preliminary subjection to a temperature of 44° C. did not affect their viability. Spores produced by a strain from white pine grown on prune agar were used in these temperature experiments. Two tubes of prune agar were melted, cooled to 36° C., inoculated with spores; from these tubes drops were transferred to van Tieghem cells. The cells were placed in duplicate in the refrigerator, where the temperature averaged about 8° C., and in incubators at 22°, 27°, 30°, and 35° C. Twenty-four hours later the spores had germinated freely at temperatures of 22°, 27°, and 30°, fairly freely at a temperature of 35° C., and not at all at 8° C. At 27° and 30° C. the germinated spores developed most rapidly producing branched mycelia 280-290μ in length. At 22° C. the mycelia were branched as before but were not more than 190 μ in length, while at 35° C. the longest hyphæ were 80-90 μ and unbranched. After 48 hours the mycelia had continued their rapid development at 22°, 27°, and 30° C., the drops had dried up at 35° C., and the spores had not germinated at 8° C. At the end of six days the spores in the refrigerator had germinated freely and in that time produced mycelia equivalent in size to those produced in 24 hours by spores at a temperature of 22° and 27° C. Thus a temperature of 8° C. greatly retarded germination, one of 22°-30° C. gave both good germination and good mycelial development, while one of 35° C. caused a noticeable retardation in mycelial growth.

D. LIGHT

Spores of *Fomes pinicola* from a fruit body produced on *Picea mariana* were sown in hanging drops of sterile distilled water and placed in a window where they received an hour or two of direct sunlight a day. Four days later most of the spores had produced germ tubes and many of them developed hyphæ 230 μ and longer. Simple septa were present but no clamp-connections were formed, and no further development took place, due no doubt to the deficiencies in the substratum.

E. GERMINATION

There is nothing unusual about the germinating spores of *Fomes pinicola* (plate II, figs. 1-11). The spores swell somewhat and may become septate before the one to four hyaline germ tubes are produced from the ends or sides of the oval, hyaline spore. These germ tubes soon become almost as wide as the original spore, and elongate rapidly producing a branched or unbranched, septate

or non-septate, hyaline mycelium. Sometimes a large much branched mycelium may develop before a single septum is formed, again a short, simple mycelium may have several septa, and both forms may occur in the same drop. If branches are formed the branching is irregular and at any angle to the main hypha. Clamp-connections are numerous in all the drops where several spores have germinated and developed. They do not appear, however, until after the mycelia have been growing for four days or more, and it has not been possible so far to determine whether or not the first one appeared after the fusion of hyphæ from two different mycelia.

Frequently the young developing mycelium produces peculiar short non-septate branches which have four or five other short branches arranged either fan-wise or in a whorl radiating from the central branch. These outgrowths are present before the production of clamp-connections and hence are probably characteristic of a primary mycelium. Helicoid hyphæ are not uncommon and chlamydospores (plate II, figs. 19-21) are sometimes formed, particularly in hanging-drop cultures that have been set up for some time. In one instance duplicate cultures were set up in van Tieghem cells and left for a month in the refrigerator at 8° C. At the end of that time the contents of the hyphæ had become aggregated into thick-walled chlamydospores of all shapes. From the appearance of the empty mycelium it was obvious that helicoid hyphæ, simple septa, and clamp-connections had been formed freely. In this instance even the secondary mycelium had become rounded up into spores.

IX. SOURCE OF CULTURES

Seventy-five cultures of *Fomes pinicola* from ten different hosts have been used in these studies. For the sake of brevity and convenience the following culture numbers will be used throughout.

Culture Numbers Used in this Bulletin

Culture No.	Source
158	Mycelium from a sporophore which grew on an old stump (*Tsuga?*).
160	" " " " *Larix laricina.*
219	" " " " *Picea* sp.
225	" " " " wood of *Picea* sp.
267	" " " " dead *Picea mariana.*
268	" " " " " "
269	" " " " " "
270	" " " " " "
271	" " " " " "
272	" " " " " "
273	" " " " " "
274	" " " " " "
280	" " " " " "
281	" " " " " "
282	" " " " " "
283	" — — — " "
283A	" " " " " " (283).
284	" — — — " "
284A, B, C, D, E	" " " " " " (284).
285	" — — — " "
285 A, B, C, D, E	" " " " " " (285).
286	" — — — " "
286 A, B	" " " " " " (286).
300	" " " " " *Picea canadensis.*
301	" " " " " wood (Sp.).
302	" " " " " *Pinus Strobus.*
303	" " " " " *Picea canadensis.*
304	" " " " " *Populus grandidentata.*
305	" " " " " *Betula alba.*
306	" " " " " *Pinus Strobus.*

CULTURE NUMBERS USED IN THIS BULLETIN—*Concluded*

Culture No.	Source
508 A, B, C	Mycelium from 3 sporophores which grew on old stump (*Tsuga*?).
509A, B, C, D, E	" 5 " " " "
510A, B, C	" 3 " " " "
511A, B	" 2 " " " "
545	" " log.
558	" " dead *Tsuga heterophylla*.
559	" " " " "
562A, C	" 2 " " " " (562).
563A, B, C, E	" 4 " " " " (563).
574A, B, C	" 3 " " " " (574).
586	" a "
635	Mycelium from a sporophore which grew on old log *Fagus grandifolia*.
694	" " " " *Pinus* sp.
5655	Mycelium from a sporophore which grew on *Populus grandidentata*.
5657	" " " " *Betula alba*.
5769	" " " " *Pinus Strobus*.
5770	" " " " *Picea mariana*.
5772	" " " " *Larix laricina*.
5575	" " " " *Abies balsamea*.
5776	" " " " "
5778	" " " " *Prunus serotina*.
158-1—158-2	2 monosporous mycelia-spores from culture No. 158
283-1—283-23	23 " " " " 283
285A-1—285A-28	28 " " " " 285A
285B-1—285B-14	14 " " " " 285B
562C-1—562C-20	20 " " " " 562C
586-1—586-26	26 " " " " 586
5770-1—5770-15	15 " " " " 5770
5778-1—5778-16	16 " " " " 5778

X. PRODUCTION OF SPOROPHORES IN CULTURES

A. ON PLANT DECOCTIONS

1. *On Plant Decoction Agars.*—The four cultures of *Fomes pinicola* 158, 160, 219, and 225 were grown on slants of bean, carrot, celery, cornmeal, malt, parsnip, potato dextrose, and prune agars in diffuse light and at room temperature. At intervals of 5 days the mycelial mat was measured, and its texture and colour noted, together with the presence of drops of liquid, zonation, or any peculiarities.

In general the development of the aerial mycelium was similar on all the agars. After 5 days a loose or compact growth of delicate, white, velvety or downy mycelium had covered the inoculum and grown out 3-5 mm. on the agar surface. After 10 days the mycelium had covered one-third to one-half of the slant with the same downy-cottony growth, which was white but on the older areas around the inoculum tinted with pale pinkish buff. From that time the colour gradually faded leaving a white mat of dense, closely felted hyphæ. This mat separated readily from the substratum and then, in both appearance and texture, resembled white glazed kid.

The influence of the substratum on the texture, colour, and rate of growth of the mycelium was particularly noticeable in the following cases. On the carrot and celery agars the four cultures grew rapidly, producing a dense felty-cottony mat. On beet agar they grew more slowly, producing a thin subfelty mat without the usual colour. Similarly on cornmeal agar the aerial growth of every mycelium was reduced to a thin white appressed-downy layer.

Some differences in the colour, texture, and amount of growth produced by each of the four cultures of *F. pinicola* were evident. Fifteen day old cultures

of 158, 160, and 219, when grown under the conditions described, exhibited the characteristic pale pinkish buff colour on bean, celery, malt, parsnip, potato dextrose, and prune agars; the mycelium remained white, ivory yellow, or cartridge buff on cornmeal, carrot and beet. Culture 255, on the other hand, showed tints of pale pinkish buff on celery, of ivory yellow on prune and carrot, and remained pure white on the other agars of the series. The mycelial mat of this culture was less dense and more powdery than those of the other three. In most cases culture 160 grew more rapidly than 158, this in turn grew more rapidly than 219 or 225. It is interesting to note that culture 219 came from a sporophore, and culture 225, which produced the white powdery mat, from the decayed wood on which the sporophore grew, yet the two differed in the colour and texture of the mycelia which they produced.

Although all the cultures in this series were grown under the same conditions of light and temperature only some of them became zoned and only some of them exuded drops of liquid. Culture 158 became zoned on malt and prune agar; culture 160 on carrot, malt, and prune, with a suggestion of zonation on celery; culture 225 became zoned on parsnip; and culture 219 gave only a suggestion of zonation on carrot and prune. Drops were exuded by culture 158 on bean, celery, cornmeal, and parsnip; by 219 on bean and celery; by 225 on bean and malt, and not at all by strain 160.

Although the series of cultures was kept for three months only two sporophores were obtained; one was produced by a culture of 158 (plate III, fig. 1), the other by 219 (plate III, fig. 3), and both on prune agar. Six weeks after the culture of 158 had been set up the mycelium on the inoculum began to grow again forming a dense, closely felted mass of white hyphæ. This growth continued until the globular mass 13 cm. in diameter projected 6 mm. from the agar surface. Gradually the outgrowth became pale pinkish buff in colour and so stood out in contrast to the remainder of the mat which had faded to a pure white some weeks previously. A few pores then opened on this surface. They developed from the interior outwards and when mature were exposed by the rupture of the very thin layer of mycelium which covered them. Twenty-four hours later there were more than double the number of pores. The sporophore was turned downward and during the night a small deposit of pure white spores was formed on the tube. At this time the whole pored surface was moist looking, and had deepened in colour to a pinkish buff (plate III, fig. 1). Spores were shed for several days, judging by the increasing density of the spore deposit. The spores from this deposit germinated readily in prune agar, producing small white mycelia. Three weeks later the colour had faded from the sporophore and a thin layer of white mycelium had grown out over it, closing many of the pores entirely. The other sporophore was entirely resupinate; the pored surface was much larger and the pores almost lamelloid (plate III, fig. 3). As the figure shows spores were shed freely by this sporophore. The first signs of mycelium growing over the pores are also visible.

2. *On Vegetable Cylinders.*—Celery stalks and cylinders of potato, carrot, and turnip, were placed in test-tubes containing a few cc. of distilled water, sterilized, and inoculated with strains 158, 160, 219, and 225, of *F. pinicola.* A luxuriant growth of dense, white, downy, and later, felted mycelium grew out from the inoculum and completely enveloped the various substrata. As before the cultures were grown in diffuse light and at room temperature, but no sporophores developed.

Similarly dried prunes were placed in a 200 cc. flask, soaked in distilled water, sterilized in flowing steam, and inoculated with strains of *F. pinicola,* but in this case growth was entirely inhibited. Prunes, like sugar beet plugs,

probably supply a too concentrated substratum. Of the latter Ferguson (19) states, "The writer has rarely succeeded in obtaining good growth with any Basidiomycete on the sugar beet as a solid substratum although almost perfect germination occurs in a decoction of the beet."

3. *On Prune Agar.*—Strains 158, 160, 219, and 225, of *F. pinicola* were inoculated in quadruplicate on tubes of prune agar in order to compare the mycelial mats produced, and to obtain sporophores from the other strains if possible.

The general course of development of all mycelia was the same. First a downy white mat which gradually gave place to a felted pale pinkish buff one, and that in turn faded to a cream or white as the mycelium became more compact and felted (plate III, figs. 1, 2, 3). But, as in the previous experiment, there were slight differences in rate of growth, texture, and colour; 158 and 160 grew more rapidly than 219 and 225; 158 developed the heaviest mycelial growth, 219 the next, and 225 the least. Strains 158 and 160 developed the usual pale ochraceous salmon colour, 225 only ivory buff, while 219 developed a light vinaceous cinnamon colour. These differences in colour were constant for all mycelial mats of each series. Strain 160 was unique in producing a naphthalene yellow colour in older cultures; a character which has been found constant throughout all later work.

After about 5 weeks the mycelia of strains 158, 160, and 219 showed signs of renewed growth. Seemingly the old hyphae grew out again producing a close, velvety, ivory yellow growth which covered the lower half of the cultures from the base of the tube to the inoculum. This became thicker and thicker until the lower half of the tube was practically filled with mycelium. In this growth, too, strain 160 produced a decided naphthalene yellow colour. Strain 225 remained unchanged.

At the end of two months every culture of strains 158, 160, and 219 produced a sporophore. Those of strain 158 developed as a rather large pinkish buff outgrowth on the inoculum and all shed heavy deposits of spores (plate III, fig. 1, 2). Strain 219 produced a different type of sporophore. The mycelium had become denser over an area of about 22 x 50 mm. This became light pinkish cinnamon in colour and pores formed over the whole area (plate III, fig. 4). Strain 160 fruited less readily and produced sporophores similar to those of 158 but much smaller (plate IV, figs. 6-8). Spores were shed by all of these fruitbodies. Strain 225 neither developed sporophores nor even thickening layers, but remained white and powdery.

To sum up, strain 158 grew rapidly, became pale ochraceous salmon, and produced bracket-like sporophores; strain 160 grew almost as rapidly, produced a thinner mycelial mat, besides the pale ochraceous salmon later developed a definite naphthalene yellow, and fruited tardily, producing small sporophores; strain 219 grew more slowly than the first two, produced a light vinaceous cinnamon colour, and large resupinate sporophores. Strain 225 grew slowly, produced a thin powdery mycelium white to ivory yellow in colour and did not fruit at all under the conditions of this experiment.

Strains 5655, 5657, 5769, 5770, 5772, 5775, 5776, and 5778, all of which had been used by Dr. Fritz in her culture studies (21) were inoculated, in duplicate, on tubes of prune agar and grown in the light at room temperature to induce sporophore formation. Table I contains a summary of the details of colour production and sporophore development by these strains and also by strains 158, 160, 219, and 225 which were discussed above.

TABLE 1.—VARIATIONS IN COLOUR PRODUCTION AND SPOROPHORE FORMATION WHICH OCCURRED IN CULTURES OF TWELVE STRAINS OF *Fomes pinicola*

Culture No.	Source	Mycelium and sporophores		
		33 days	40 days	70 days
5655	*Populus grandidentata*	White powdery	White powdery	White powdery, *no sporophores.*
5657	*Betula alba*	Pinkish buff, pores forming.	*Sporophores*	Naphthalene yellow
5769	*Pinus Strobus*	Pale pinkish buff and pinkish buff.	*Sporophores* (vinaceous fawn).	Naphthalene yellow.
5770	*Picea* sp.	Pale pinkish buff. *Sporophores*	Ivory-naphthalene yellow.	
5772	*Larix laricina*	White, powdery.	White, powdery	Naphthalene yellow, *pores forming*
5775	*Abies balsamea*	Pinkish buff	*Sporophores* (vinaceous buff).	Tints of naphthalene yellow.
5776	*Abies balsamea*	Pale pinkish buff and pinkish buff.	Pale pinkish buff, powdery.	*No sporophore.*
5778	*Prunus serotina*	Pale pinkish buff and pinkish buff.	Tints of naphthalene yellow, pores forming.	*Sporophores.*
158	*Tsuga* (?)	Pale pinkish buff	Pale pinkish buff	*Sporophores.*
160	*Larix laricina*	Pale pinkish buff	Pale pinkish buff	*Sporophores.*
219	*Picea* sp.	Light vinaceous cinnamon.	Light vinaceous cinnamon.	*Sporophores.*
225	*Picea* sp.	White, powdery	White, powdery	White, powdery, *no sporophores.*

Sporophores were formed on mycelia originating from *Betula alba, Pinus Strobus, Picea* sp. (2 strains), *Larix laricina, Abies balsamea, Tsuga* (?), and *Prunus serotina* (plate III, figs. 6-10); but three strains, one from *Abies balsamea,* one from *Populus grandidentata,* and one from *Picea* remained sterile throughout this experiment.

Sporophores were eventually obtained from all mycelia except that from poplar (5655). The strain from larch (5772) fruited very tardily, but on prune agar under the usual conditions (plate IV, fig. 5). Cultures 5655 and 225 (spruce) were grown in large tubes (2 by 12 inches) containing 150 cc. of prune agar in the light and at room temperature. At the end of three months 225 developed a pored area, but 5655 never fruited although it was left for six months.

The delayed sporophore production in culture 225 can scarcely be attributed to host influence, for other strains from spruce (219, 5770) fruited normally and readily. On the other hand both strains from larch (5772 and 160) fruit tardily and produce small, almost abnormal sporophores in culture (plate IV, figs. 6-8). In this connection it is interesting to note that, although tints of naphthalene yellow are frequently present in older cultures of most strains, this colour appears much earlier and is much more pronounced in the strains from larch.

4. *On Prune Decoction.*—500 cc. of prune decoction were placed in each of eight 2-litre flasks, sterilized intermittently, inoculated with small pieces of mycelium-covered agar from a culture of 158, and grown in the light at room temperature. A thin almost colourless mass of submerged mycelium developed about the inoculum and grew slowly until the surface of the liquid was reached. Then it spread rapidly covering the surface with a thin mat. This gradually thickened, became downy and later cottony-felty in texture and pale pinkish buff in colour. After some months every flask contained small masses of mycelium which had grown out from the mat, increased in size until some extended 3

cm., became a moist pinkish buff in appearance and developed normal pores. The mats were several times as thick as but otherwise similar in texture and appearance to those which fill the crevices of wood which has been badly decayed by *F. pinicola*.

B. ON CZAPEK'S SYNTHETIC MEDIUM

1. *Solidified.*—Strains 158, 160, and 219 of *Fomes pinicola* were grown in quadruplicate on Czapek's synthetic medium solidified with agar and on two modifications of it in which (1) arabinose was substituted for the dextrose of the formula, and (2) peptone was substituted for the inorganic nitrate (medium 7).

On Czapek's medium, growth was similar for the three strains (plate III, fig. 5). A thin, appressed, spreading mycelium grew out from the inoculum and gradually covered the agar surface, the older growth becoming white and light downy in texture. Later small, irregular, flocculent masses of denser mycelium developed, particularly in 219. The cultures remained white except that small areas of pale pinkish buff developed in 160 and a small sporophore of the same colour developed on one of the denser areas in 219.

Growth on the medium containing arabinose was as rapid as that on Czapek's and similar to it except that 219 developed the white, downy growth in only two areas, 158 over but half the culture, and 160 scarcely any at all. On this medium as on the previous one small but dense masses of white mycelium which became pored developed in cultures of 219.

The substitution of peptone changed the colour and type of growth entirely. Here dense, downy mycelium developed immediately and spread rapidly, becoming light pinkish cinnamon then tilleul buff to vinaceous buff in colour, and gradually felted. Drops of amber-coloured liquid were exuded by 158 and 160, and marked zonation was present in culture 158. Eventually the mycelial mat became entirely felted, faded out to cartridge buff and white, but no sporophores were produced.

Strain 158 was inoculated in duplicate on tubes of Czapek's agar and on prune agar. The fungus grew as rapidly on one as on the other, producing in each case the typical growth previously described—on Czapek's thin, appressed to light-downy, white; on prune dense, downy-felty, pale pinkish buff to pinkish buff. After two months the mycelium on prune agar produced a sporophore measuring 2·7 x 1·8 x ·5 cm. and pinkish buff in colour. One week later a sporophore similar in colour and measuring ·8 x ·8 x ·4 cm. grew out from the inoculum on Czapek's agar (plate III, fig. 5). With one exception this is the largest and most normal sporophore of *F. pinicola* obtained on Czapek's synthetic medium.

2. *On plain agar.*—Because the aerial mycelium of *F. pinicola* developed so poorly on Czapek's agar, cultures of this fungus were grown on distilled water solidified with 2·5 per cent agar, for purposes of comparison. Strain 158 was inoculated in triplicate on tubes and 120 cc. flasks containing this plain agar. A thin, colourless, appressed mycelium developed and spread over the agar surface. It was similar in general appearance to that on Czapek's but thinner, and with only suggestions of downy growth and no sporophores.

3. *Czapek's Synthetic Medium (liquid).*—A series of 200 cc. Erlenmeyer flasks was set up in duplicate, each flask containing 50 cc. of Czapek's synthetic medium in which the following substances had been substituted for the usual 3 per cent dextrose as a source of carbon: 2 per cent dextrose, levulose, lactose,

maltose, raffinose, inulin, soluble starch, amygdalin, xylose, arabinose, pectin; 4 per cent butyric acid, olive oil; and checks with no substitute for the dextrose. The flasks were sterilized intermittently and then inoculated with minute pieces of aerial mycelium which had been removed from a two week's old culture of strain 158 of *F. pinicola* grown on prune agar. Prior to inoculation 3 drops of 5 unit insulin were added to three of the flasks containing levulose, maltose, and dextrose respectively.

Growth was entirely inhibited in the flasks containing butyric acid, and was very slow in the others. It consisted of a small mass of loosely interwoven hyphæ which developed a thin, powdery, scattered, aerial mycelium, white in colour. The cultures containing insulin with levulose, maltose, and dextrose, and the one containing pectin were the only ones in which this mycelial mat covered even half the surface of the medium; in the ones containing soluble starch and lactose the aerial mycelium was still less, in the remainder the aerial growth was limited to a very small mat or was entirely absent as in the check cultures.

With this medium, as with Czapek's agar, sporophores were produced although the amount of aerial mycelium was so limited. Fruit-bodies developed in the flasks containing levulose, lactose, maltose, raffinose, soluble starch, xylose, arabinose, and pectin, as indicated in the table which follows:—

Table II.—Summary of Mycelial Growth and Sporophore Production in Flask Cultures of *Fomes pinicola*

Czapek's synthetic medium containing	Number of sporophores	Size of sporophores	Submerged mycelium	Aerial mycelium
1. Dextrose	0		Scant	1·5 cm. diam.
2. Dextrose + insulin	0		4 times as much as in (1).	"
3. Levulose	0		As in check	None
4. Levulose + insulin	1	6 mm. diam	"	1·5 cm. diam.
5. Lactose	1	4 mm	Dense, 2·3 cm. diam	Scant
6. Maltose	1	6 x 8 x 4 mm	Scant	Scant
7. Maltose + insulin	1	7 x 10 x 20 mm. 2nd pore layer 13 x 15 x 20 mm.	Dense	2 sq. cm.
8. Raffinose	2	3 mm. diam	1·5 cm. diam	Scant
9. Inulin	0		Scant	Scant
10. Soluble starch	2	3 mm. diam	Scant	1·5 cm. diam.
11. Amygdalin	0		Scant	Scant
12. Xylose	1	3 x 4 mm	Scant	Scant
13. Arabinose	1	2·5 mm. diam	As in check	Scant
14. Pectin	0		As in lactose	2·5 cm. diam.
15. Butyric acid	0		None	None
16. Olive oil	0		As in check	Scant
17. Check	0		Scant	Scant

The influence of insulin on both mycelial development and sporophore production was marked. In the medium containing dextrose the fungus developed four times as much submerged mycelium in the presence of insulin as in its absence; in the medium containing levulose a sporophore developed in the flask containing insulin and none in its absence; and in the medium containing maltose a dense mass of submerged mycelium, a large aerial mat and a very large sporophore, which eventually produced a second pore layer, developed in the presence of insulin, while a scanty growth of aerial and submerged mycelium and a very much smaller sporophore developed in the flask without it. However, such experiments, to be conclusive, require definite confirmation.

C. ON WOOD BLOCKS

(*a*) *Small.*—Blocks of the following wood were prepared and sterilized in the way already described:—

1. *Abies balsamea*
2. *Acer saccharum*
3. *Betula papyrifera*
4. *Carya ovata*
5. *Castanea* sp.
6. *Fagus* sp.
7. *Fraxinus americana*
8. *Fraxinus nigra*
9. *Juglans cinerea*
10. *Juglans nigra*
11. *Larix americana*
12. *Lyriodendron*
13. *Picea canadensis*
14. *Pinus contorta*
15. *Pinus divaricata*
16. *Pinus palustris*
17. *Pinus ponderosa*
18. *Pinus racemosa*
19. *Pinus Strobus*
20. *Platanus* sp.
21. *Populus tremuloides*
22. *Prunus serotina*
23. *Pseudotsuga taxifolia*
24. *Quercus alba*
25. *Quercus nigra*
26. *Quercus rubra*
27. *Robinia pseudo-acacia*
28. *Sassafras variifolium*
29. *Thuja occidentalis*
30. *Tilia* sp.
31. *Tsuga canadensis*
32. *Ulmus americana*
33. *Ulmus fulva*
34. *Ulmus racemosa*

The wood blocks were then inoculated with a small piece of mycelium-covered agar from a culture of *Fomes pinicola* which came originally from spruce (219). The tubes were placed in trays and slanted at an angle of about 15° and grown in diffuse light at room temperature.

The fungus failed to grow on *Thuja occidentalis* but on all the other woods it developed a thin silky downy growth about the inoculum. At the end of one month all showed varying amounts of a thin, spreading, downy-flocculent to powdery mycelium covering from one-third to the whole face of the wood block.

From that point development proceeded in one of three ways: (*a*) the mycelium gradually disappeared from the outside of the wood-block, e.g. *Ulmus fulva, Platanus, Sassafras, Thuja occidentalis,* and *Larix americana;* (*b*) it remained thin and scattered while areas of varying size consisting of denser, felted to powdery mycelium developed on the surface of the block, e.g. *Castanea, Quercus rubra, Populus tremuloides* (plate V, fig. 7), and *Acer saccharum* (plate V, fig. 9); or (*c*) it developed dense, felted mats which partially or wholly enveloped the block and frequently entirely filled the test-tube, e.g. *Prunus serotina, Tilia* (plate V, fig. 5), and *Ulmus racemosa* (plate V, fig. 6).

When, seventeen months after inoculation, the blocks of wood were removed from the tubes and examined, all the characters of the typical *F. pinicola* rot were found. Usually decay was indicated by the rough uneven surface left by the saw in cutting and by the fractured appearance of the wood, as in charcoal, which, in advanced stages of decay, was friable; frequently the decayed wood was darker in colour though this character was not constant, due, no doubt, to the colour changes which had taken place previously during the preparation and sterilization of the blocks. In several instances typical mycelial sheets were found filling up the crevices of the decayed wood.

The amount of aerial mycelium produced in a culture gave no real indication of the amount of decay in the wood block. Some wood blocks without any mycelium on the outside were almost completely rotted, e.g. *Pinus Strobus* and *Fraxinus nigra;* others were apparently sound, e.g. *Thuja occidentalis* and *Platanus;* some with scattered areas of aerial mycelium were decayed only beneath that mycelium, e.g. *Pseudotsuga taxifolia* and *Robinia;* others were almost wholy decayed, e.g. *Pinus ponderosa;* some with dense enveloping aerial

mycelium showed only a thin layer of the outer wood decayed, e.g. *Ulmus racemosa* (plate V, fig. 6); while others were so badly decayed that they were held together only by the mycelium which surrounded them, e.g. *Tilia* (plate V, fig. 5).

It is striking that in every case the dense mycelial mats which enveloped the wood occurred on wood blocks from deciduous trees: *Acer saccharum, Betula alba, Carya ovata, Juglans cinerea, Populus tremuloides, Prunus serotina, Robinia, Tilia, Ulmus americana,* and *U. racemosa.* The nearest approach to such development on coniferous wood occurred on the block from *Pinus divaricata* (plate V, figs. 1-4) where the mat was much less dense than those on deciduous woods. Why such mats should only develop on deciduous woods is not clear, for on *Castanea, Fagus, Fraxinus americana, Juglans nigra,* and *Sassafras* the fungus grew and rotted the wood but without any such development. Except when the wood was covered by a layer of dense mycelium, it was usually a part or the whole of the interior of the block which was rotted, while the outer layer remained more or less intact.

Some attempt at sporophore development was obtained on all the wood blocks except those of *Abies balsamea, Fagus* sp., *Lyriodendron tulipifera, Pinus palustris, P. ponderosa, Platanus, Thuja occidentalis,* and *Ulmus racemosa.* Pored sporophores were developed on the following woods; *Betula alba, Carya ovata* (plate V, fig. 8), *Juglans cinerea, Larix americana, Picea canadensis, Pinus contorta, P. divaricata* (plate V, figs. 1-4) *Populus tremuloides* (plate V, fig. 7), *Sassafras variifolium, Tilia* sp. (plate V, fig. 6), and *Tsuga canadensis.* The failure of many of the rudimentary sporophores to develop normal pored surfaces was, in all probability, due to the drying out of the culture. They were all moistened with sterile water three times during the seventeen months but during the intervals some dried out much more rapidly than others. Among those sporophores listed as rudiments in Table III are several large and well developed masses of the typical *F. pinicola* context, e.g. on *Acer saccharum* (plate V, fig. 9), which under suitable conditions would probably have developed normally.

In all cases the sporophore was white at first and remained white or pale pinkish buff until development was complete. Later some became naphthalene yellow, others various shades of buff. No lacquered or hardened outer surface developed on any sporophore in culture but, as was said before, the context of the fruit-bodies was typical both in texture and colour and readily recognizable as that of *F. pinicola.*

The sporophores produced on wood blocks were much varied both in size and shape. Some were almost globular, some elongated and flat, one which grew on the upper surface of the block was shortly but very definitely stipitate (plate V, fig. 9). The largest sporophore developed on the wood of *Carya ovata,* 22 by 22 by 7 mm. (plate V, fig. 8), but the most interesting one on the wood of *Pinus divaricata.* Four months after the culture had been inoculated a spherical mass of mycelium, ivory yellow to pale pinkish buff in colour, grew out from the wood, developed a pored surface and shed its spores (plate V, figs. 1-2). It gradually faded to white and then became a dull buff in colour, identical with that found in older layers of sporophores produced in the field. During the next three months a layer of white mycelium grew out and covered the under surface of the sporophore. It gradually became thicker, developed a pored surface and shed spores (plate V, fig. 3). A third pore layer was developed in the same way during the next month (plate V, fig. 4), and when the culture was finally discarded, seventeen months after it had been inoculated, a fourth layer of mycelium had begun to develop. As far as the writer is aware this is the first time that the development of a sporophore of this type in culture has been reported.

The mycelial development, sporophore formation, and the decay which occurred on the various kinds of wood are summarized in the table which follows:

TABLE III—*Fomes pinicola* (219) ON WOOD-BLOCK CULTURES

On Wood of	Type of Mycelium		Number of sporophores	Decay
	6 months	12 months		
Abies balsamea	2 small areas, drops, white, powdery.	No change	O	
Acer saccharum	Small areas on each side of block, white, powdery.	Very dense, block ½ covered.	1 stipitate 8x8x4mm. rudiment.	No visible rot except small area on one side.
Betula alba	3 sides of block covered, white, felted, dense.	Block ⅚ covered, very dense.	2 pored 15x8x4 mm. 14x12x4 mm.	Advanced stage, friable beneath mycelial layer.
Carya ovata	4 sides half covered, white, felted, dense.	Block ½ covered, very dense.	1 pored 22x20x7 mm.	Advanced stage of rot.
Castanea sp.	Small area, thin, downy, white.	Block ½ covered, thin, downy-powdery.	1 rudiment 4x7 mm.	Small amount charcoal-like, friable.
Fagus sp.	Areas—white, thin, downy.	Block ½ covered, white, downy.		Small amount charcoal-like, friable.
Fraxinus americana	Small area, very thin, powdery.	No change	1 rudiment, 2 layers, 10 x 7 x 2 mm.	Block rotted except outside layer.
Fraxinus nigra	Small area, very thin, powdery.	No change	1 rudiment, 2 layers, 10 x 4 x 2 mm.	Centre of block in advanced stage.
Juglans cinerea	Block covered, thin, powdery-subfelty, white, pale pinkish buff.	½ block, dense	1 pored, 18 x 8 x 4mm	Advanced stage, centre of block only.
Juglans nigra	2 sides — thin, powdery, drops.	Small areas, thin, powdery.	1 rudiment, 6 x 6 x 3 mm.	Centre of block rotted.
Larix americana	3 very small areas thin, white, powdery.	None	1 pored, 10 x 8 x 6 mm.	Small amount in advanced stage.
Liriodendron tulipifera.	1 side only, thin, powdery.	1 small area, powdery.	O	No visible rot.
Picea canadensis	3 sides, downy, white	Small area, thin powdery.	1 pored, 5 x 4 x 2 mm.	Small amount advanced.
Pinus contorta	No aerial mycelium	Very small area, thin, downy.	1 pored, 6 x 5 x 3 mm.	Advanced stage of rot interior of block.
Pinus divaricata	Areas of spreading, downy-powdery.	Block ⅓ covered, dense, downy.	1-14 mm. diam. globular 3 pore-layers	Block rotted, friable advanced stage.
Pinus palustris	Small area, white, powdery.	No change	O	No visible rot.
Pinus ponderosa	3 sides thin, flocculent, downy.	Block ⅓ covered, white, downy.	O	Part of block badly rotted.
Pinus racemosa	Small area, thin, powdery.	None	3 rudiments, 1, 2 x 2 mm., 2 rudiments, 15 x 7 x 2 mm.	Interior of block only, advanced rot
Pinus Strobus	Practically none	None	1 rudiment, 3 mm. diam.	Most of block friable
Platanus	Small area, thin, downy.	None	O	No visible rot.
Populus tremuloides.	⅛ covered, dense, felted, white.	No change	1 pored, 15 x 11 x 9 mm.	Small area rotted.
Prunus serotina	½ covered, dense, felted, white.	⅚ covered dense, felted, white.	2 rudiments, 4 x 4 x 3 mm., 8 x 8 x 4 mm.	Centre of block rotted.
Pseudotsuga taxifolia.	Small area, white, downy.	Small area, white, powdery.	1 rudiment, 2 layers, 8 x 5 x 4 mm.	Small amount, typical.
Quercus alba	Traces of thin, downy mycelium.	Very small area, thin, powdery.	1 rudiment, 7 x 3 x 2 mm.	Very small amount.
Quercus rubra	Small area, powdery, ivory yellow.	Small area, dense to powdery.	Rudiment, 8 x 4 x 3 mm.	Small amount near inoculum, friable.
Robinia sp.	⅔ covered dense, felted, white.	No change	1 rudiment, 11 x 10 x 4 mm.	Typical rot beneath dense mycelium.
Sassafras variifolium.	2 sides, very thin, powdery.	None	1 pored, 18 x 7 x 7 mm.	Centre of block in advanced stage of rot.
Thuja occidentalis	No growth	No growth	O	No rot.
Tilia sp.	3 sides dense, downy white.	¾ block, very dense.	1 pored, 20 x 11 x 6 mm.	Almost completely rotted.
Tsuga canadensis	Small area, downy.	No change	1 globular pored, 7·5 mm. diam.	Advanced stage, wood soft, friable, mycelial sheets.
Ulmus americana	3 sides, thin, spreading.	Very dense	1 rudiment, 4 x 4 x 2 mm.	Part of block rotted.
Ulmus fulva	Traces, thin, spreading.	None	1 4 x 4 x 3 mm.	No rot visible.
Ulmus racemosa	⅚ covered, very dense, white felted.	No change	O	Decay around outside of block only; just beneath mycelium.

I am very much indebted to Dr. J. H. White of the School of Forestry, University of Toronto, for his kindness in supplying the material used in this experiment.

B. LARGE WOOD BLOCKS

Blocks of *Tsuga heterophylla* 2 by 2 by 4 inches were prepared, placed obliquely in 2-quart jars upon layers of water-soaked cotton, plugged, and sterilized. A square of mycelium-covered agar from a culture of *Fomes pinicola* was then placed upon the upper transverse surface. The cultures were grown in the light and at room temperature, and were moistened with sterile water at intervals of three months.

A thin, spreading, downy-silky, white mycelium grew out from the inoculum and gradually covered part of the upper transverse surface and grew slowly over parts of the lower sides of the blocks. This mycelium later became sub-felty to powdery in texture, but otherwise no further change occurred until one year after the inoculations had been made.

At that time sporophores began to develop, appearing first as minute hemispherical masses of white mycelium which gradually increased in size and sometimes coalesced. There were ten of these sporophore rudiments on one block (plate X, fig. 2) six on the upper transverse surface and two on each of two sides; on the other block there were five, all of which were much elongated in shape and occurred on the two under sides of the block. They varied in size from 4-13 mm. in diameter and the largest measured 24 by 15 by 6 mm. When sectioned these mycelial masses exhibited the typical *F. pinicola* context, corky in texture with tints of massicot yellow. The blocks were not moistened again, and no pores developed.

When the blocks were removed nineteen months after they had been inoculated they were found to be perfectly dry and, except for shrinkage which was evident from the presence of several large longitudinal cracks, there was no outward sign of decay. When they were split open, however, both blocks were found to be rotted from top to bottom (plate X). The mycelium had spread rapidly in a longitudinal direction and gradually outward so that the central part of the blocks was almost completely destroyed. Except for a very thin layer at the surface the wood was much darker in colour and had contracted in both directions producing longitudinal and transverse cracks which were filled with masses of white hyphæ. The wood did not splinter but broke off sharply, and was very friable. In fact all the characters of the typical *F. pinicola* rot were present and the red brown wood with charcoal-like fracture held together with sheets of white mycelium did not differ in any way from wood of *Tsuga heterophylla* rotted by the same fungus under natural conditions.

XI. EFFECT OF VARIATIONS IN TEMPERATURE AND ACIDITY UPON MYCELIAL DEVELOPMENT

A. TEMPERATURE

Prune agar slants were inoculated from a 25-day-old culture of *Fomes pinicola* (158) and grown, in quadruplicate, at the following temperatures: (*a*) in a refrigerator (6°-10° C.); (b) in incubators at 22°, 29°, 32°, 35° C.; and (*c*) in the light at room temperature. Observations were made and recorded at weekly intervals and these are summarized in table IV. From this it is evident that variations in temperature caused differences in the rapidity of mycelial growth and in the texture and colour of the mycelium developed.

The influence of temperature upon rapidity of mycelial growth was very definite. *F. pinicola* grew very slowly at a temperature of 6°-8° C., rapidity of growth increased with the temperature up to about 29° C.; from that point an

increase in temperature resulted in a decrease in growth, and a temperature of 35° C. became almost inhibitive (plate IV, figs. 1-4). At first the mycelial mats produced at 29° C. and 32° C. were similar in texture but by the end of the second week those at 29° C. were the most luxuriant, those at 32°C. next, 22° C. next, then 35° C. and finally those at 6°-8° C. and at room temperature. Colour appeared first in the cultures at 32° C., then in those at 35° C., and at room temperature. It was deepest in those cultures grown at 32° C. and 35° C., fairly deep in those at room temperature, and very faint in those grown at 29°, 22°, and 8° C.

The influence of temperature upon the rate of growth of the mycelium of *Fomes pinicola* is similar to that found by Fritz (21) for *F. igniarius, F. roseus, F. fomentarius,* and *P. Schweinitzii;* its influence upon colour production in this form, i.e. intensification of colour by increase of heat, is similar to that found for *F. igniarius, F. roseus* and *Lenzites sepiaria.* It was also found by Fritz that diffuse light caused a deepening of colour, and the colour present in the mycelia grown at room temperature and in the light is just another instance of that fact.

No sporophores were produced, even in the light, by any of these cultures of *Fomes pinicola* during the time they were under observation. However, the cultures grown at a temperature of 6°-8° C. in the refrigerator were left there for almost eight months. At the end of that time no apparent change had occurred; the cultures were not dried up, but no sporophores had been produced. They were removed then and placed in the light at room temperature and two weeks later two of them had produced pinkish buff, pored areas. Since Long and Harsch (34) have obtained sporophores of *F. pinicola* in cultures grown in the dark it would seem that the low temperature and not lack of light inhibited sporophore development in this instance.

The table which follows summarizes the results of experiments on the influence of temperature upon the texture, rate of growth, and colour production in cultures of *F. pinicola.*

TABLE IV.—INFLUENCE OF TEMPERATURE ON MYCELIAL DEVELOPMENT IN CULTURES OF *Fomes pinicola*

Days	6°–8°C.	Room	22°C.
7	No growth	12 x 18 mm., thin appressed, white.	22 x 36 mm., downy, white.
14	Short downy growth on inoculum.	22 x 45 mm., downy-felty	22 x 68 mm. downy-silky to woolly-felty.
21	8 mm. diameter, downy-silky, white	22 x 62 mm., thin silky-downy to felty, tints of pale pinkish buff.	Medium covered, very dense, downy-felty, drops.
28	20 x 22 mm., downy-silky, white	Medium covered thin, felty, pale pinkish buff.	Downy growth around sides of tubes, white.
35	20 x 40 mm., thin, downy, pale pinkish buff on inoculum.	Pale pinkish buff to pinkish buff.	Tints of pale pinkish buff.

Days	29°C.	32°C.	35°C.
7	22 x 50 mm. dense, downy-cottony, white.	22 x 30 mm., very dense, downy silky, white.	Thin downy growth on inoculum only.
14	Medium covered, very dense, woolly-felty.	22 x 46 mm. dense, downy-felty.	22 x 25 mm., fairly thin, cottony.
21	Felted, except base of tube filled with downy-felty growth.	Medium covered close, felty, drops, pale pinkish buff.	22 x 40 mm., dense, downy, white.
28	Downy growth around sides of tubes, tints of pale pinkish buff.	Close felty, pale pinkish buff.	Felted, pale pinkish buff to pinkish buff.
35	No change	Pale pinkish buff to pinkish buff.	Drying out.

B. ACIDITY

Fomes pinicola grows so well in culture on a number of media, untitrated, that only one experiment was made to test the effect of the initial acidity of the medium upon mycelial development. Czapek's synthetic liquid medium (modified) was brought to a pH value of 4·2, 4·8, 5·2, 5·8, and 6·2, by the addition of monobasic or dibasic potassium phosphate. 75 cc. of each medium were placed in each of six 200 cc. Erlenmeyer flasks. Three of each series were inoculated with *F. pinicola* mycelium which had been isolated from *Picea* (219), the remaining three of each series were inoculated with mycelium isolated from *Abies balsamea* (5776). All of the cultures were grown in diffuse light at room temperature.

After two weeks the mycelial growth was still entirely submerged and consisted of a more or less spherical, whitish, translucent mass of hyphæ around the inoculum—a mass not more than 3 cm. in diameter, and usually much less. It was quite evident, however, that the greatest amount of growth occurred in the media with a pH value of 4·8 and 5·2, and the least in that with a pH value of 6·2.

After four weeks the masses of mycelium had increased in size but were still entirely submerged, except that a small amount of aerial mycelium had developed in the flasks containing media with a pH value of 4·8 and 5·2.

The cultures were kept under observation during four months. At the end of that period the media in all the flasks with the exception of that with an original pH value of 6·2 had become pale amber in colour. There was slightly more aerial mycelium in the flasks containing media with an original pH value of 4·2, 4·8, and 5·2, but the amount of growth in all cultures was practically equal. Since fungous growth usually causes a change in the acidity of the medium it may be that in those cultures with a high initial acidity a point was reached where further growth was inhibited, while in those which were originally more nearly neutral, growth could continue for a longer period, giving finally a similar result in all flasks.

The pH value of the medium at the end of the experiment was not determined, and, since *F. pinicola* does not grow well in liquid media the experiment was not repeated. However, it does seem definite that *F. pinicola* prefers an acid medium, as Rumbold (55) found, and preferably one with a pH value of 4·8-5·2.

XII. VARIATIONS IN CULTURAL CHARACTERS AND IN THE FORMATION OF A LINE OF DEMARCATION IN MIXED CULTURES

A. VARIATIONS IN CULTURAL CHARACTERS OF MYCELIUM FROM DIFFERENT SOURCES

Throughout the description of cultures of the mycelium of *F. pinicola* frequent references have been made to the variations which occur in rate of growth, in amount and texture of mycelium, in colour, in zonation, and in sporophore production when comparative cultures are made of mycelia from various sources.

These differences in cultural characters are, apparently, due to individual variations rather than to host influence. At least it has not been found that one type of mycelium is isolated, constantly, from one type of fruit-body, from one host, or from different hosts in one locality.

To study variations in cultural characters fifty-seven cultures of *F. pinicola* including 158-306, and 5655- 5778, together with a number of monosporous and polysporous origin, were sown in quadruplicate on prune agar, and grown in an incubator at 22·5° C. At the end of seven days the mycelium was uniformly downy with practically no appressed advancing zone; the average amount of

growth in all tubes was 31·7 mm. Culture 5772 (from *Larix*) and 280 (*Picea*) grew most rapidly; 5655 (*Populus*) was much denser and silkier in texture than the average; 282 (*Picea mariana*) produced by far the densest mat; while 284 C. (*P. mariana*) gave the least growth, producing a thin, appressed to powdery mycelium. *F. pinicola* does not produce much colour on prune agar at any time, and still less in the absence of light. However, there were traces of colour in 302 (*Pinus*) and in six cultures from *Picea*.

At the end of ten days the medium in most tubes was three quarters or completely covered with mycelium. Growth was much denser than the average in cultures 268 and 282 (*P. mariana*). Zonation was present in cultures 267, 274, 280, 281, 283, 283A, 284, 284B, 284B ++, 284D, and 285B (all from *P. mariana*) and in 305 (*Betula alba*). Colour was entirely absent from cultures 269, 270, 282, 283, 283A, 284A ++, 284D, 285C, 285E ++, and 287 (all from *P. mariana*), and from 160 and 5772 (*Larix*), and from 302 (*Pinus*). Varying amounts of pale pinkish buff, pale ochraceous salmon, pinkish buff, and light pinkish cinnamon were present in the remaining cultures. Colour was deeper than the average in 219, 268, and 271 (*Picea*), and especially in 300 (*Picea*), which was uniformly pinkish buff throughout the culture. Culture 284 (*Picea*) still gave the least growth, while 5655 (*Populus*) was unique with its pure white mycelium, uniformly dense and velvety. 282 (*Picea mariana*) resembled it somewhat, but the advancing zone was more downy-silky, and the remainder somewhat felted.

When the cultures were 18 days old they were divided into the following groups and examined for similarities and differences: (1) cultures from sporophores from the same host species; (2) cultures from sporophores from a single tree; (3) cultures from sporophores and from the spores they produced; (4) cultures alike in macroscopic characters.

1. *Cultures of F. pinicola from sporophores which grew on the same host species.*

(*a*) on *Larix laricina*. (160 and 5772).—The cultures from both sources were so nearly alike that it was impossible to separate them by mycelial differences (plate IV, figs. 4-8). The mycelium was pure white, dense, downy, and later irregularly felted.

(*b*) on *Picea mariana*. (267-286).—In this series of 148 cultures from 37 sources there was no culture quite as unique as 5655 (*Populus*), but there were all gradations from the slow-growing, thin, powdery, mycelium such as 284C, to the rapidly spreading, dense, downy-felty mycelium of 280, which gave next to the best growth of any culture. There were similar gradations in colour; cultures 282, 284D, and 285D were pure white, while 286, 286A, and 268 were deeply coloured. It is obvious, then, that in this series from *Picea mariana* there is no definite type of culture associated with this host.

(*c*) on *Picea canadensis*. (287, 300, 303).—287 produced a very dense, downy growth, white, with the merest trace of colour. 300 produced a much thinner growth, felted, and exhibiting the deepest colour of any culture. 303 produced a mycelium with the texture of 300 but with the colour of 287.

(*d*) on *Populus grandidentata*. (304, 5655).—These two cultures were absolutely different in every respect, the mycelium of 5655 was pure white in colour, dense, uniform, velvety in texture, and showed definite zonation; 304 was pale pinkish buff, with points of pinkish buff and cinnamon buff at the base, of moderate thickness, downy to tufted, felted in some cases, and without any sign of zonation.

(*e*) on *Pinus Strobus*. (302, 306).—These two cultures were alike in texture, both were white with tints of pale pinkish buff, but 306 was slightly denser and had developed more pale pinkish buff at the tip of the culture.

(*f*) on *Betula papyrifera*. (305, 5657).—These two cultures were similar in texture, downy and irregularly felted, but the colour was much deeper in 305.

(*g*) on *Abies balsamea*. (5775, 5776).—Culture 5775 was thin, downy to subfelty, and white in colour; 5776 was denser, felted, and white tinted with pale pinkish buff.

The evidence presented shows that cultures from sporophores growing on one species of host do not, necessarily, or even usually, resemble one another very closely; i.e. although there are many different types of cultures it has not been possible to associate one type with cultures from any one host.

2. *Cultures isolated from several different sporophores which grew on one tree and from the infected wood of the tree itself.*

All of the material used in this series came from *Picea mariana* and was collected at Timagami, Ont., by Dr. Faull.

(*a*) Cultures 283 and 283A, from infected wood and from the context of a sporophore which grew upon it. The cultures in these two series were identical in every way, markedly so in the presence in both series of two denser zones of mycelium, corresponding, probably, to two exposures to sunlight.

(*b*) 284 (from infected wood), 284A, B, C, D, E, (sporophores which grew on 284). Culture 284, 284B, and 284D were identical in every way, downy in texture, and showing two zones of mycelium which was denser and somewhat more deeply coloured than the rest. 284A resembled this group in texture, but grew more slowly. 284C was unique. It grew very slowly, producing a thin, subfelty mycelium. 284E produced a mycelium which was denser than the others and deeper in colour, while zonation was almost entirely absent.

(*c*) 285A, B, C, D. All of these cultures were somewhat similar, white in colour, or only faintly shadowed with pale, pinkish buff, downy in texture, and of moderate thickness; yet they were distinct enough that the twenty tubes could be sorted into their respective groups without much difficulty. Cultures of 285B showed zonation, those of 285C were more evenly downy and grew around the sides of the tubes, those of 285D were denser and more felted, those of 285 were felted, but not as dense as 285D.

(*d*) 286 (from infected wood), 286A and 286B (from sporophores which grew on 286). 286 and 286A were indistinguishable, producing a heavy, downy to woolly growth, and a very deep colour. 286 B was similar in texture but denser, and with no hint of colour.

Again, the evidence from comparative cultures shows that even mycelia from a number of sporophores from one tree, and from the infected wood of that tree do not, necessarily, resemble one another very closely.

3. *Cultures isolated from several different sporophores and from the spores which they produced.*

(*a*) 268 and 268 ++ (spores from 268). These two mycelia were practically identical; they could be distinguished only by the fainter colour at the bases of the tubes containing 268 ++.

(*b*) 269 and 269++. These two mycelia were identical, uniformly downy-felty, and white.

(*c*) 282 and 282 ++. These two mycelia were entirely different. 282 was pure white, woolly-felted in texture, and very dense; 282 ++ was tinted with pale pinkish buff, downy, and not so dense.

(*d*) 283A and 283A ++. These two mycelia were identical in texture, fairly thin, and subfelty, but 283A was pure white, 283A ++ was uniformly pale pinkish buff.

(*e*) 284B, and 284B ++. These two mycelia differed mostly in texture, 284B was less dense, and more uniform in texture than 284B ++, which was almost woolly and definitely zoned.

(*f*) 285A, and 285A++. These two mycelia were practically identical in texture and colour.

Hence, the mycelium obtained from sporophore context may differ from that obtained from spores from that sporophore in colour, in density, or in texture, or in all three; though in many cases the mycelia from two such sources resemble one another closely.

It has been shown that mycelia from sporophores from the same host species, or from sporophores from the same tree, or from sporophores and the spores which they produced, need not resemble one another closely in culture. Some idea of the amount of variation in cultures of *F. pinicola* may be gleaned from plate VIII, figs. 5-8. Fig. 5 illustrates a mycelium isolated from a sporophore which grew on *Populus grandidentata.* The growth is dense, and uniformly felted. Fig. 6 illustrates a mycelium isolated from *Pinus Strobus* and shows a dense, uniformly cottony-woolly mycelium which has begun to grow around the sides of the tube. Fig. 7 represents a mycelium isolated from *Pinus* sp. (New Brunswick), and shows a thin, even, downy mycelium; while fig. 8, isolated from *Tsuga heterophylla* (Vancouver, B.C.), shows a downy mycelium with irregular, thickened areas of woolly mycelium, which became much denser at the tip of the culture. On the other hand, figs. 1-4 show mycelia with a fairly uniform, downy growth of moderate density, yet (1) was isolated from *Tsuga heterophylla,* Vancouver, B.C., (2) from *Pinus* collected in New Brunswick, (3) from *Tsuga heterophylla* collected on Queen Charlotte Islands, B.C., and (4) from *Picea canadensis,* Timagami, Ontario. In this group, then, there are mycelia from localities as far removed as Vancouver, B.C., Timagami, Ont., and New Brunswick, yet the cultures are quite similar.

Since, then, it has not been found that one type of mycelium is isolated, constantly, from one type of fruit-body, from one host, or from different hosts in one locality, the differences in cultural characters have been considered to be due to individual variation, rather than to the influence of any specific factor. Yet, as Dr. Fritz (21, p. 225) pointed out, these differences are not great enough to render it impossible to identify *F. pinicola* in culture, with a fair degree of accuracy. Observations on the rate and mode of growth, colour production, sporophore formation, and the size and types of hyphae produced in culture, combine to give a very fair means of determining this species despite the variations.

B. MIXED CULTURES AND THE FORMATION OF A LINE OF DEMARCATION

A great deal of work has been done by Zeller and Schmitz (78), Brown (8), Porter (49), and others on mixed cultures of fungi, but their interest lay, primarily, in the behaviour of fungi when different species, genera, or families, were mixed. Thus Schmitz and Zeller worked with such forms as *Lenzites, Merulius, Daedalea, Trametes, Pleurotus,* and *Polyporus:* Brown used organisms representative of all the great fungous groups.

Brown (p. 126) states that, "The question of the intermingling or non-intermingling of two fungal colonies (of the same or different species) is intimately connected with the question of staling. . . . The phenomenon is not one of absolute incompatibility, and investigation would probably show that any fungus could be made to intermingle with any other under appropriate conditions." Porter describes in detail five types of reaction which occur when fungi are grown in the presence of other fungi: type A mutually intermingling,

not common; type B, growth superficial over the contending organism; type C, slight inhibition, ("This is the prevailing type when any organism is grown with another individual of the same species"); type D, growth around the contending organism; type E, mutual inhibition at a considerable distance. He suggests (P. 174) three possible explanations for the morphological changes which occur in mixed cultures: "(*a*) The nutrients may be exhausted. (*b*) The distortions may be due to change in the osmotic equilibrium of the medium induced by the metabolic activities of the growth process. (*c*) Certain poisonous products may be created by fungous growth capable of producing malformations and creating a zone through which fungous filaments can not pass." He demonstrates, among other things, that inhibitions are not so marked in media rich in nutriments, that they vary but slightly with changes in the amount of inoculum, in time of inoculation, or in depth of medium, and that a "common cause of the inhibitory action in the cases studied was determined to be the presence of some product formed during growth."

Schmitz (57), in working with strains of *Fomes pinicola*, found that there was no intermingling when four cultures of this fungus from four different hosts, Douglas fir, white fir, western hemlock, and western white pine, were paired with one another.

Cayley (11), working with *Diaporthe perniciosa*, goes still further and finds mutual aversion between monospore mycelia. She finds that " the phenomenon of aversion between two antagonistic strains has occurred on all media so far used irrespective of the depth of the medium, the distance at which inocula are placed or the age of the cultures when tested." Aversion was found usually to occur "between mono-mycelia from different hosts whether of the same variety, different varieties, or different species, but mono-mycelia isolated from such widely different hosts as apricot and plum have been found to meet; on the other hand averting strains have been found in different perithecia on the same host, thus showing that the type of strain is irrespective of the variety of host. It is quite possible that the occurrence of averting strains on the same host may be due to multiple infection from two or more different sources, and not to the splitting up into physiological strains in the host plant."

Smith (62) notes an interesting fact, namely, the presence of black lines in some crustaceous lichens. "A patch of crustaceous lichen on tree or rock may belong to one species and yet be composed of many individuals which have started from different centres, each growing centrifugally. The dark lines chiefly occur when the different individuals encounter each other. A striking instance of such intersecting lines occurs in the thallus of the well-known *Rhizocarpon geographicum*. Strong boundary lines also frequently divide different species inhabiting the same substratum."

In view of these interesting results it was thought worth while to extend Schmitz' studies of mixed cultures of *Fomes pinicola*. With the material available it was possible to test the effect on one another of organisms from many different hosts, or from the same host growing in the same or different localities, of organisms from the same individual tree, and of organisms from single spores from the same sporophore. Some experiments were made as well to test the effect of variations in temperature, amount of light, and concentration of nutrients in the medium, on the behaviour of paired cultures. Unless otherwise stated all paired cultures were made on Petri plates 10-12 by 1-1·2 cm. containing a layer of malt agar about 0·5 cm. thick. The small pieces of mycelium used as inocula were placed about 1·5 cm. apart and near the centre of the plate. Cultures were incubated at 25° C. for from 15 to 20 days.

(1) *Various Hosts and Various Localities.*—In this series all possible pairings of seventeen different cultures of *F. pinicola* were made, using cultures from the following sources:—

Culture Number	Host	Locality
305	*Betula alba*	Timagami, Ontario.
635	*Fagus grandifolia*	Chelsea, Quebec.
5778	*Prunus serotina*	York Mills, Ontario.
304	*Populus grandidentata*	Timagami, Ontario.
5775	*Abies balsamea*	Guelph, Ontario.
160	*Larix laricina*	Guelph, Ontario.
5772	*Larix laricina*	Guelph, Ontario.
300	*Picea canadensis*	Timagami, Ontario.
303	*Picea canadensis*	Timagami, Ontario.
286A	*Picea mariana*	Timagami, Ontario.
694	*Pinus* sp.	St. John, New Brunswick.
302	*Pinus Strobus*	Timagami, Ontario.
5769	*Pinus Strobus*	Guelph, Ontario.
306	*Pinus Strobus*	Guelph, Ontario.
558	*Tsuga heterophylla*	Vancouver, B.C.
509	*Tsuga heterophylla*	Queen Charlotte Island, B.C.
586	?	France.

The results are given in table V where the sign + indicates the presence of a definite line between the two mycelia and sign 0 the complete intermingling of the two mycelia.

TABLE V.—ALL POSSIBLE PAIRINGS OF SEVENTEEN CULTURES (DIPLOID) OF *Fomes pinicola* FROM VARIOUS HOSTS AND VARIOUS LOCALITIES

—	305	635	5778	304	5775	160	5772	300	303	286A	694	302	5769	306	558	509	586
305	0	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
635	+	0	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
5778	+	+	0	+	+	+	+	+	+	+	+	+	+	+	+	+	+
304	+	+	+	0	+	+	+	+	+	+	+	+	+	+	+	+	+
5775	+	+	+	+	0	+	+	+	+	+	+	+	0	+	+	+	+
160	+	+	+	+	+	0	0	+	+	+	+	+	+	+	+	+	+
5772	+	+	+	+	+	0	0	+	+	+	+	+	+	+	+	+	+
300	+	+	+	+	+	+	+	0	+	+	+	+	+	+	+	+	+
303	+	+	+	+	+	+	+	+	0	+	+	+	+	+	+	+	+
286A	+	+	+	+	+	+	+	+	+	0	+	+	+	+	+	+	+
694	+	+	+	+	+	+	+	+	+	+	0	+	+	+	+	+	+
302	+	+	+	+	+	+	+	+	+	+	+	0	+	+	+	+	+
5769	+	+	+	+	0	+	+	+	+	+	+	+	0	+	+	+	+
306	+	+	+	+	+	+	+	+	+	+	+	+	+	0	+	+	+
558	+	+	+	+	+	+	+	+	+	+	+	+	+	+	0	+	+
509	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	0	+
586	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	0

From the table it is obvious that, with two exceptions, 160 by 5772 and 5769 by 5775 (plate VI, figs. 4, 3), complete intermingling of mycelia occurred only when the two inocula came from the same culture, that is when a culture was paired with itself. The mycelial growth in such plates is just as uniform as if it had originated from a single inoculation (cf. plate VI, figs. 3, 4). The remainder, always excepting 160 by 5772, and 5769 by 5775, showed inhibition in every case; a line formed by a dense growth on the part of one, (plate VI, fig. 1) or, more frequently, both of the mycelia (plate VII, figs. 5, 6) developed where the two mycelia met. In these pairings cultures were used from ten different host trees, some deciduous, some coniferous; from the same host but different localities; and from the same host and the same locality; yet all of the cultures remained distinct and showed antagonism to every other culture.

In order to obtain further proof that, despite the behaviour of 160 and 5772 and 5769 and 5775, inhibition occurred when cultures were obtained from the same host species and the same locality, all possible pairings were made of 35 mycelia of *Fomes pinicola*, all from *Picea mariana* and collected at Timagami, Ontario.

Cultures 267-282 isolated from sporophores on different trees.

Cultures 283-286 isolated from infected wood of different trees.

Cultures 283A, 284A-E, 285A-D, 286A-B isolated from each of a group of sporophores on tree 283, tree 284 etc.

Cultures 268 ++, 269 ++, etc., cultures from spores shed by sporophores 268, 269, etc.

The results of pairing these cultures are embodied in table VI, where the same signs are used as in table V, with addition of the minus sign — which indicates slight inhibition resulting in a space between the two mycelia, but no line (plate VII, figs. 3, 4).

TABLE VI.—ALL POSSIBLE PAIRINGS OF THIRTY-FIVE CULTURES (DIPLOID) OF *Fomes pinicola* FROM ONE HOST SPECIES, *Picea mariana*, AND ONE LOCALITY, TIMAGAMI, ONT.

—	267	268	269	270	271	272	273	274	280	281	282	283	283A	284	284A	284B	284C	284D	284E	285	285A	285B	285C	285D	286	286A	286B	268++	269++	282++	283++	284A++	284B++	285A++	285E++
267	0	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
268	+	0	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	0	+	+	+	+	+	+	+
269	+	+	0	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	0	+	+	+	+	+	+
270	+	+	+	0	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
271	+	+	+	+	0	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
272	+	+	+	+	+	0	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	−	+	+	−	+	+
273	+	+	+	+	+	+	0	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
274	+	+	+	+	+	+	+	0	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
280	+	+	+	+	+	+	+	+	0	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
281	+	+	+	+	+	+	+	+	+	0	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
282	+	+	+	+	+	+	+	+	+	+	0	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
283	+	+	+	+	+	+	+	+	+	+	+	0	0	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	0	+	+	+	+
283A	+	+	+	+	+	+	+	+	+	+	+	0	0	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
284	+	+	+	+	+	+	+	+	+	+	+	+	+	0	−	0	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	−	0	+	+
284A	+	+	+	+	+	+	+	+	+	+	+	+	−	0	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	0	+	+	+
284B	+	+	+	+	+	+	+	+	+	+	+	+	0	+	0	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	−	0	+	+
284C	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	0	+	+	+	+	+	+	+	+	+	+	+	+	+	+	−	+	+	+
284D	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	0	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
284E	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	0	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
285	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	0	+	0	+	+	+	+	+	+	+	+	+	+	+	+	+
285A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	0	+	+	+	+	+	+	+	+	+	+	+	+	0	0
285B	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	0	+	0	+	+	+	+	+	+	+	+	+	+	+	+	+
285C	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	0	+	+	+	+	+	+	+	+	+	+	+	+
285D	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	0	+	+	+	+	+	+	+	+	+	+	+
286	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	0	0	+	+	+	+	+	+	+	+	+
286A	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	0	0	+	+	+	+	+	+	+	+	+
286B	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	0	+	+	+	+	+	+	+	+
268++	+	0	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	0	+	+	+	+	+	+	+
269++	+	+	0	+	+	−	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	0	+	+	+	+	+	+
282++	+	+	+	+	+	+	+	+	−	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	0	+	+	+	+	+
283++	+	+	+	+	+	+	+	+	+	0	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	0	+	+	+	+
284A++	+	+	+	+	+	+	+	+	+	+	+	+	+	−	0	−	−	+	+	+	+	+	+	+	+	+	+	+	+	+	+	0	−	+	+
284B++	+	+	+	+	+	−	+	+	+	+	+	+	+	0	+	0	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	−	0	+	+
285A++	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	0	+	+	+	+	+	+	+	+	+	+	+	+	+	0	+
285E++	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	0	+	+	+	+	+	+	+	+	+	+	+	+	+	+	0

From table VI it is obvious that a line of aversion is the usual reaction obtained when mycelium from sporophores or wood from various individuals of the same host species, and even from the same locality, are paired in culture. It will be noted that complete fusion between the two mycelia takes place only under the following conditions:

(*a*) Whenever a mycelium is paired with itself.

(*b*) When mycelium from sporophore tissue is grown with mycelium from spores produced by the sporophore. A space or a white line of dense growth

may occur under such circumstances. In plate IX, figure 2 shows mycelium 158 paired with a mycelium obtained by germinating a number of spores from a sporophore produced by culture 158, i.e., a polysporous mycelium; while figure 3 shows culture 158 (upper and larger mycelium), paired with two monosporous mycelia derived from spores shed by culture 158. There is an absence of aerial mycelium where the two monosporous mycelia meet, yet they form clamp-connections whenever they are paired.

(*c*) Usually when mycelium from infected wood of a tree is grown with mycelium from a sporophore which grew upon the tree.

(*d*) Usually when mycelium from spores or sporophore tissue is grown with mycelium from spores or sporophore tissue from a second sporophore from the same tree.

In all pairings recorded in the table fusion has never taken place between any but closely related mycelia, since the mycelium obtained from spores and that from the sporophore which produced them, or mycelium from infected wood and that from the sporophore which grew upon it would, ordinarily, be considered identical. The fact that mycelium from wood (284) does not pair with that from three of the sporophores which grew upon it, i.e. 284C, 284D, 284E, would indicate that several infections had taken place, a conclusion corroborated by the results obtained in all possible pairings of monosporous mycelia of 285A and 285B (table IX). In the light of these results the complete fusion which occurs in every paired culture of 160 x 5772 or 5775 x 5769 is most unusual.

160 and 5772 were each isolated from a sporophore which grew on larch. Both were collected at Guelph, Ontario, but 160 was collected a year and a half later than 5772. As will be seen in plate VI, fig. 4, the mycelia are indistinguishable. Both develop a decided naphthalene colour in the aerial mat and both produce sporophores very tardily (plate IV, figs. 5-8). It is possible that both cultures came from sporophores which grew on the same tree. And, since no other case has been found where mycelia from two different host species fuse, there might be a vague possibility that either 5769 and 5775 were infected from the same source, or else that infection spread from one to the other.

(2) *Pairings of Monosporous Mycelia.*—Since no explanation of the formation of a line of aversion could be found either in a study of mycelia from various host species from the same or different localities, or in a study of mycelia from numerous individuals of the same species, the behaviour of paired monosporous mycelia was investigated. All possible pairings of 10 monosporous mycelia from each of four sources, i.e. 283, 586, 5770, and 5778, were made, and the behaviour of the paired mycelia recorded. It was found that lines of aversion were common in these cultures despite the fact that the spores, in each case, came from one sporophore. The line of aversion was white, yellow, brown, or black. If the two mycelia united to form clamp-connections the line was either absent (plate IX, fig. 3), or else white or yellow, or, occasionally, brown in colour. No black line was ever found in these pairings except between two mycelia which did not form clamp-connections (cf. Vandendries, 71).

An interesting point is that, despite these results, when monosporous mycelia from one geographical race of *F. pinicola* are paired with monosporous mycelia from any other geographical race, clamp-connections result (see section XIII, D) yet frequently a very definite line of demarcation is formed at the meeting of the two mycelia.

(3) *Effect of Variations in Cultural Conditions upon the Formation of a Line of Demarcation.*—Porter (49) found that inhibitions are not so marked on media rich in nutriments, and that they vary only slightly with changes in amount of inoculum, in time of inoculation, or in depth of medium. Brown (8),

on the other hand, considered that, in all probability, any fungus could be made to intermingle with any other fungus, under suitable conditions. The behaviour of paired cultures of *Fomes pinicola* was tested on media rich and poor in nutrients, and on thick and thin plates, under various conditions of light and temperature. In these experiments only those pairs were used whose behaviour under conditions adopted as standard was well known; i.e. when grown on plates of malt agar in an incubator at 25° C. The following media were used: washed agar, peptone agar, Czapek's synthetic agar, with, or without, the addition of dextrose, peptone, or both; prune, malt, and bean agars, potato agar, with the addition of glycerine or dextrose. The results are tabulated below where the sign 0 indicates the absence of any line of demarcation, and the plus sign + indicates the presence of a very definite line.

TABLE VII.—EFFECT OF VARIOUS MEDIA UPON THE AMOUNT OF MYCELIUM AND UPON THE FORMATION OF A LINE OF DEMARCATION IN PAIRED CULTURES OF *Fomes pinicola*

Medium	Growth	5775 x 5769	286 x 286B	283 x 286B	5655 x 280	284 x 284A
Plain agar	Extremely thin aerial mycelium.	0	0	0	0	0
Czapek's agar no sugar	" "	0	0	0	0	0
Peptone agar	Very thin aerial mycelium	0	0	0	0	0
Nutrient agar	" "	0	0	0	0	0
Czapek's agar + peptone	" "	0	0	0	0	0
Czapek's agar + dextrose	" "	0	+	+	+	+
Prune agar	Fairly dense aerial mycelium.	0	+	+	+	+
Malt agar	" "	0	+	+	+	+
Bean agar	" "	0	+	+	+	+
Potato agar	" "	0	+	+	+	+
Potato agar + dextrose	" "	0	+	+	+	+
Czapek's+peptone+dextrose	Very dense aerial mycelium	0	+	+	+	+
Potato agar + glycerine	" "	0	+	+	+	+

The paired culture 5775 x 5769 could not be induced to form a line. The others, which, under standard conditions, formed heavy lines of demarcation, gave no such reaction on media poor in nutrients or unsuited to fungous growth. But on any medium where the fungus was able to produce even a thin mycelial mat the black line of demarcation developed at the meeting point of the two mycelia. The mycelial mat was very heavy on Czapek's agar with peptone and dextrose, and on potato with glycerine, and the line was correspondingly marked. Hence in paired cultures of *F. pinicola* reactions are more marked on media rich in nutrients, a result which does not agree with Porter's conclusions in that regard.

The thickness of the medium in the plates made little difference to the results obtained provided, always, that there was sufficient medium so that it did not dry up before the time required for the formation of a line, i.e. during a period of ten days or so. Plates filled as full as possible were inoculated with two mycelia, and a very heavy, dense, black line developed between the two. The use of deep plates with a thin layer of medium or shallow plates filled as full as possible did not materially affect the results, so that, within that range, variations in amount of air and moisture were negligible.

(4) *Temperature.*—A series of plate cultures of 286A x 635 and 286A x 5778, two pairs which give very definite lines of demarcation, was inoculated on malt agar and treated as follows:—

1 plate of each incubated at 25°C. in light.
1 plate of each incubated at 25°C. in darkness.
1 plate of each grown at room temperature, in light.
1 plate of each grown at room temperature, in darkness.
2 plates of each placed in refrigerator,

All cultures grew, and all produced black lines of demarcation. The mycelia developed much more rapidly in the incubator; those in the dark producing a spreading silky growth, those in the light a more downy one. At room temperature there was considerably more growth in the dark than in the light, but all cultures grew more slowly than those in the incubator. This was to be expected, for light has an inhibiting effect on the growth of many fungi and it has already been shown that the optimum temperature for mycelial growth of *F. pinicola* is 24°-28°C. The line of demarcation developed first in the cultures in the incubator and, in both series, developed in cultures kept in the dark before it appeared in those growing in the light.

Growth was exceedingly slow in cultures kept in the refrigerator. After twenty-one days the mycelia were not more than an inch and a half in diameter, and the aerial mycelium had been checked on either side of the line of meeting, leaving a noticeable space between the two. One month after inoculation the mycelia had not completely covered the plates but a very definite black line of demarcation had developed in every culture, and the usual drops of brownish liquid were being exuded in culture 286A x 635.

Hence, though variations of light and temperature may have a very decided influence on the rate of formation, they do not inhibit the development of a line of demarcation.

(5) *Diffusion of metabolic products.*—In order to see whether the formation of such a line of demarcation was due to diffusion of by-products of metabolism beyond the zone of fungous growth, a number of plates were inoculated with culture 283, and a number with 282. When the mycelia were about two inches in diameter a 1-inch square of agar was removed from the outer edge of culture 282, taking with it about $\frac{1}{4}$ inch of the mycelium. A similar square was removed from culture 283, and the two squares were interchanged, placing the mycelial growth away from the centre of the plate. Thus, if inhibition were due to diffusion of metabolic products, a black line should form where mycelium of 283 met agar from culture 282, and vice versa. However, no line was formed until the hyphæ themselves met. Duplicate series gave the same results.

Again, a series of plate cultures was made, using four inoculations, two of culture 282 and two of 283, placing the two likes side by side, or diagonally opposite each other. The placing of the inocula made no difference whatsoever. Complete fusion occurred whenever the mycelium of 282 met 282 and 283 met 283 and a line of demarcation developed, wherever 282 met 283. Other experiments in which the inocula were placed close together, or far apart, or where one inoculation was made several days after the other, showed that such variations had no effect on the final result. A line of demarcation formed where the two mycelia met.

(6) *Discussion.*—When two mycelia of *Fomes pinicola* are grown together in Petri plate cultures various reactions are obtained. These may be grouped roughly into types A, C, D, and E of Brown (8). Type A, mutually intermingling, is not common, except between two mycelia which are closely related in origin. Type C, slight inhibition, is the usual reaction, as Brown has stated. This slight inhibition manifests itself in various ways: (1) mutual inhibition of aerial growth, resulting in a narrow space between the two mycelia (plate VII, figs. 3, 4). The aerial mycelium may develop into a thick mat on either side of this space (plate VII, fig. 2). (2) Formation of a line of demarcation. This may be white, consisting of a heavier mycelial growth along the line; or brown, or black, due to the actual discoloration of the hyphae in that area. The agar beneath the line is frequently darkened. In cases where a very dense black line is formed drops of a dark brown liquid, acid in reaction, are exuded fre-

quently (plate VII, fig. 1). Type D, growth around the contending organism, occurs only when one mycelium grows much more slowly than the other. In that case one grows completely around the other but a space of aversion or a line of demarcation usually results as well. The line of demarcation which develops at the line of contact of two antagonistic mycelia of *Fomes pinicola* is jet black, tough, and almost sclerotial-like in character. At first it is just a dense growth of the white submerged mycelium, which later becomes yellow in colour, and finally brown and black. Sections show that the black layer extends the full depth of the mycelial growth and is rather like a very narrow wall dividing one mycelium from the other. A tough, white, aerial mycelium frequently develops on the agar surface directly above the black line and is frequently coterminous with it.

The dark colour is due to the browning of the hyphae at the line of meeting. The contents of the cells in that area are coarsely granular and range from a yellow-brown to a deep brown in colour (plate II, fig. 26), but whether or not they are dead has not been determined. The walls do become brittle and break up under a pressure that leaves the ordinary hyaline hyphae unchanged. In an attempt to prove that the hyphae were dead small squares were cut from the tough black line formed in paired cultures and placed on malt agar plates and in hanging drops in van Tieghem cells. A typical dense white mycelium developed from each inoculum, and every attempt to find new hyaline hyphae developing from the brown discoloured ones of the inoculum was unsuccessful. Mycelium might have developed from normal hyaline hyphae that were present, no doubt, in the inoculum. Rhoads (51, p. 43) when studying the black zones formed by wood-destroying fungi found that, in wood "the decomposition products arise only after the death of the cells through the oxidation of their contents and certain constituents of their walls," and he intimates that oxidation of the contents of living cells does not occur and hence that a black line forms only after the death of the cells. By analogy the same interpretation might be made here but proof is still wanting.

From the various experiments described above it is concluded that the formation of a line of aversion between two mycelia of *Fomes pinicola*, when grown together, is not due to host influence. It develops when mycelia from different genera and species of deciduous and coniferous hosts are paired, when mycelia from the same host species but from different localities are paired, and when mycelia from the same host species and the same locality are paired. It may even develop when mycelia from two sporophores growing on the same tree are used. Nor is the formation of a line of aversion in cultures of *F. pinicola* dependent upon variations in temperature, light, or amount or kind of medium, provided that one is used with sufficient available nutrients to produce a normal mycelial development.

Nor is the formation of a line of demarcation in paired cultures of two monosporous mycelia of *F. pinicola* an infallible indication that the two mycelia have remained in the haploid condition. To obtain further evidence upon this point some two hundred paired cultures were made, using for the first time culture 831 which was isolated from a sporophore collected on *Betula papyrifera* at Timagami, Ont. The cultures which were inoculated on malt agar plates as usual, and incubated at 25° C. for four weeks, included:—

Series (1). All possible pairings of nine monosporous mycelia of culture 831.

Series (2). All possible pairings of five monosporous mycelia from 831 with five monosporous mycelia from each of the cultures used in previous experiments viz: 283, 285A, 285B, 562C, 586, 928, 5770, and 5778. Each pairing was examined for the presence of a line of demarcation and for clamp-connections with the following results:

(*a*) Series (1). Although a line of demarcation never developed in pairings of two monosporous mycelia of 831 except when they remained in the haploid condition, yet the mycelia might remain in the haploid condition without any line of demarcation being formed.

(*b*) Series (2). In the pairings of monosporous mycelia of 831 with monosporous mycelia of all the other cultures, *except 562C*, lines of demarcation were formed in from three to seven of the twenty-five pairings in each series, yet clamp-connections were formed in every pairing; that is a line of demarcation may be, and is found, occasionally, even though the two monosporous mycelia have united to form a diploid mycelium.

(*c*) Series (2). In the pairings of monosporous mycelia of 831 with monosporous mycelia of 562C a line of demarcation was present in only fifteen of the twenty-five pairings yet the mycelia remained in the haploid state in every pairing of this series. Hence it seems that the formation of a line of demarcation between two monosporous mycelia is not entirely dependent upon the sexual reaction of these two mycelia.

No definite conclusion has been reached as to the exact conditions which lead to the formation of a line of demarcation between two monosporous or polysporous mycelia of *F. pinicola*. However, since Schmitz (57) has shown that physiological variation does exist in the mycelium of this fungus it seems probable that in paired cultures the effect of one mycelium upon another at the line of meeting causes an upset in metabolism, and that the density of the line of demarcation may be an indication of the amount of disturbance.

XIII. HETEROTHALLISM IN *FOMES PINICOLA*

In 1918 Mlle Bensaude (4), working with *Coprinus lagopus*, proved that heterothallic species exist among the Basidiomycetes. This most interesting discovery has been corrobrated and extended through the investigations of Kniep (30-32), Vandendries (68-71), Hanna (24), Miss Newton (42-43), Brunswik (9), the author (38-39), and others. The earlier literature on the subject of sexuality in the Basidiomycetes has been adequately dealt with by Mlle Bensaude, and, since the later papers are all fairly closely correlated, it is, perhaps, only necessary here to summarize some of the results of immediate interest in connection with *F. pinicola:*—

1. Both homothallic and heterothallic species occur in the genus *Coprinus;* the heterothallic species may be either bisexual or quadrisexual.

2. There are many so-called sexual strains (or geographical races) which are perfectly cross fertile.

3. Monosporous mycelia of a bisexual species may change spontaneously from the haploid (primary) to the diploid (secondary) state.

4. A sporophore may develop on a monosporous mycelium of a heterothallic species, but the spores are all of one sex.

5. Recent work with *Coprinus micaceus* has led Vandendries (71*a*) to conclude that "Entre individus d'une même aire géographique, c'est la fertilité qui est normale. Entre souches éloignées, appartenant a deux aires géographiques distinctes, la stérilité est la règle."

So far no large wood-destroying polypore has been investigated from this point of view, hence this study of the sexuality of *Fomes pinicola* was undertaken.

Morgan (37) and Brunswik (9) both prefer to explain the behaviour of paired monosporous mycelia on the basis of self-sterility rather than on the basis of sex. Such an explanation brings these investigations more or less

into line with East's work (15) on self-sterile plants. However, in this paper, for the sake of uniformity the terms originally used have been maintained. Should it be necessary the results may be interpreted at any time in terms of self-sterility factors.

Both Mlle Bensaude and Kniep have shown that in a mycelium bearing clamp-connections the nuclei occur in pairs, that is the mycelium is in the diploid condition. Thus, the absence of clamp-connections from monosporous mycelia and the presence of clamp-connections on compound mycelia resulting from the union of two monosporous mycelia of opposite sex provides a reliable criterion for the determination of heterothallism, and it is the only one which has been used in determining that *Fomes pinicola* is heterothallic.

A. ISOLATION OF MONOSPOROUS MYCELIA

Since the mycelia of *Fomes pinicola,* isolated from wood or grown from sporophore tissue, fruit in culture, spores from material from many sources were available for the making of monosporous cultures. Unfortunately the spores are small and the walls so delicate that the dry-needle method (24) could not be used, and much difficulty was experienced in the actual technique. At first Van Tieghem cells were used and the drops inoculated in various ways. Tubes of melted agar or gelatine were inoculated with a few spores and drops removed with a platinum loop and placed on sterile cover-slips. Occasionally a drop was found which contained a single spore, but the method is unsatisfactory. A better way was to place small drops of melted agar on a coverslip, insert the coverslip beneath a sporophore and allow the spores to fall directly on the drops of agar. Eventually the method adopted was as follows: a sporophore was removed from a culture with sterile forceps and quickly fastened to the cover of a sterile Petri dish by means of a drop of melted agar. Then the cover was removed from a Petri dish containing lactose gelatine, the cover bearing the sporophore was substituted, and slowly rotated. Then the original cover was replaced and as many Petri dishes as desired were inoculated in this way. The plates were kept at room temperature for from four to five days. By that time the germinating spores appeared as tiny depressions in the surface of the clear gelatine. A circle was drawn around each mycelium, it was examined under the microscope, and if it proved to be monosporous, and if there were no other spores within the circle, it, surrounded by a small square of gelatine, was removed with a sterile spear-shaped needle, placed on a potato dextrose or malt agar slant, and grown at room temperature. In this way 163 monosporous mycelia were isolated from ten different sources as follows:—

158	Sporophore from old hemlock (?) stump, Bond Lake, Ont.	2
285A	" *Picea mariana*, Timagami, Ont.	28
285B	" same tree as No. 285A, Timagami, Ont	14
283	" *Picea mariana*, Timagami, Ont.	23
5770	" *Picea* sp., Guelph, Ont.	15
5778	" *Prunus serotina*, York Mills, Ont.	16
586	Culture from sporophore of *F. pinicola* var. *marginatus*, isolated and determined by M. Jean Dufrenoy, France.	26
562C	Sporophore from old log (*Tsuga heterophylla*?), Vancouver, B.C.	19
831	" *Betula papyrifera*, Timagami, Ont.	12
928	" *Betula* sp., Stockholm, Sweden.	8
		163

Each monosporous mycelium was labelled with the number of the culture from which it was isolated, together with a serial number, so that its origin was obvious yet its identity was maintained throughout; e.g. the numbers 285A-1, 5778-3, 586-25, indicate that the mycelia in question were the first, third, and twenty-fifth monosporous mycelia to be isolated from one spore-deposit of cultures 285A, 5778, and 586, respectively.

B. *FOMES PINICOLA* IS A HETEROTHALLIC BISEXUAL SPECIES

Malt agar in Petri plates was used for practically all the paired cultures; malt agar is quickly made, gives a very satisfactory growth, and inoculations can be made more rapidly in plate cultures than in tubes.

In pairing mycelia a small square of mycelium-covered agar, about 3-4 mm. in length, was removed from a young culture and placed near the centre of the Petri plate. A similar square was removed from the culture, with which the first was to be paired, and placed 15-20 mm. from the first inoculum. The cultures were incubated at 22°C. for from 10-14 days. Then a small square of agar was removed from the line of meeting of the two mycelia, mounted in water, and examined under the high power of a microscope. When the two mycelia formed a compound secondary mycelium clamp-connections were numerous, large, and well-formed, so that positive determinations could be made fairly rapidly.

All possible pairings were made of from ten up to twenty-six of the monosporous mycelia isolated from each spore-deposit. The results were tabulated and table VIII is typical of the results from each of the six series of pairings. The plus sign + indicates the presence of clamp-connections (plate II, fig. 25) on paired mycelia, the minus sign — indicates their absence.

TABLE VIII.—*Fomes pinicola.* ALL POSSIBLE PAIRINGS OF TWENTY-SIX MONOSPOROUS MYCELIA ISOLATED FROM CULTURE NO. 586 (FRANCE)

586

—	1	2	5	9	12	13	15	17	23	25	26	3	4	6	7	8	10	11	14	16	18	19	20	21	22	24
1	−	−	−	−	−	−	−	−	−	−	−	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
2	−	−	−	−	−	−	−	−	−	−	−	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
5	−	−	−	−	−	−	−	−	−	−	−	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
9	−	−	−	−	−	−	−	−	−	−	−	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
12	−	−	−	−	−	−	−	−	−	−	−	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
13	−	−	−	−	−	−	−	−	−	−	−	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
15	−	−	−	−	−	−	−	−	−	−	−	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
17	−	−	−	−	−	−	−	−	−	−	−	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
23	−	−	−	−	−	−	−	−	−	−	−	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
25	−	−	−	−	−	−	−	−	−	−	−	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
26	−	−	−	−	−	−	−	−	−	−	−	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
3	+	+	+	+	+	+	+	+	+	+	+	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−
4	+	+	+	+	+	+	+	+	+	+	+	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−
6	+	+	+	+	+	+	+	+	+	+	+	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−
7	+	+	+	+	+	+	+	+	+	+	+	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−
8	+	+	+	+	+	+	+	+	+	+	+	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−
10	+	+	+	+	+	+	+	+	+	+	+	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−
11	+	+	+	+	+	+	+	+	+	+	+	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−
14	+	+	+	+	+	+	+	+	+	+	+	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−
16	+	+	+	+	+	+	+	+	+	+	+	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−
18	+	+	+	+	+	+	+	+	+	+	+	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−
19	+	+	+	+	+	+	+	+	+	+	+	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−
20	+	+	+	+	+	+	+	+	+	+	+	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−
21	+	+	+	+	+	+	+	+	+	+	+	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−
22	+	+	+	+	+	+	+	+	+	+	+	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−
24	+	+	+	+	+	+	+	+	+	+	+	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−

In table VIII the mycelia have been rearranged so that those which behave alike are grouped together; e.g. mycelium 586-1 does not form clamp-connections when paired with mycelia 1, 2, 5, 9, 12, 13, 15, 17, 23, 25, and 26, from the same spore-deposit; but it does form clamp-connections when paired with mycelia 3, 4, 6, 7, 8, 10, 11, 14, 16, 18, 19, 20, 21, 22, and 24. Mycelia 2, 5, 9, 12, 13, 15, 17, 23, 25, and 26 behave in exactly the same way as 586-1. From the table it is evident that the spores from a single fruit-body fall into two groups; clamp-connections are formed only when a member of one group is paired with a member of the opposite group. The results were identical whether the original isolations were made from a deciduous host (cultures 5778, 831, or 928), or from

a coniferous host (cultures 285A, 283, etc.); and whether that host came from one of several localities in Ontario (Guelph, York Mills, Timagami), from British Columbia, from Sweden, or from France. Hence *Fomes pinicola* is undoubtedly a heterothallic species, and, since the spores from a single fruit-body fall into two groups, and only two, it is bisexual. This species is, therefore, comparable with *Coprinus radians* investigated by Vandendries (69), *C. Rostrupianus* investigated by Miss Newton (42), and *C. Friesii, C. comatus, C. velaris,* and *C. deliquescens* investigated by Brunswik. (9).

C. CULTURAL CHARACTERS AND FRUITING OF HAPLOID AND DIPLOID MYCELIA

One hundred and twenty-two monosporous mycelia of *Fomes pinicola* from five different sporophores were sown in duplicate on large slants of prune agar and grown in an incubator at 22°C. Ten days after inoculation these mycelia showed variation in amount of growth of from 17-45 mm. with an average of about 35 mm. Many of them produced a fairly thin, appressed, downy mycelium, but the majority showed the downy-woolly or, occasionally, silky mycelium of *F. pinicola.* The mats were uniform in thickness, and, even in older cultures, did not tend to become as tough as those of polysporous origin. However, these individual variations can scarcely be attributed to host influence. There is as much variation among the individual monosporous mycelia arising from spores from one sporophore as there is among polysporous or tissue cultures from the same or different hosts (see section XII).

Nor has it been possible to link up these variations in cultural characters with the behaviour of monosporous mycelia in paired cultures. For example, table IX shows the results obtained when all possible pairings of 10 monosporous mycelia from fruit-body 285A were made. Mycelia 1, 2, 4, 6, 7, and 8 belong to one group, mycelia 3, 5, 9, and 10 belong to another group; clamp-connections are produced only when a mycelium from the first group is paired with a mycelium from the second group, or vice versa. Yet, in culture, mycelia 4, 5, and 8 are practically identical. Mycelia 1, 4, 7, and 10 give a fairly dense growth, 3 and 9 give only a moderate growth, while 2 is extremely thin and spreading. Similarly, all possible pairings of mycelia 1-6 from sporophore 285B show that mycelia 1, 3, and 4 belong to one group and 2, 5, and 6 belong to another group. Yet in culture mycelia 1, 2, and 4 produce a very dense mat, mycelia 3 and 5 produce a very thin spreading one, and mycelium 6 is intermediate. Or again, all possible pairings of mycelia 4-9 from sporophore 5770 show that mycelia 4, 6, 7, and 8 belong to one group and 5 and 9 to another, yet in culture the mycelia are indistinguishable. And all possible pairings of mycelia 1-6 from sporophore 283 show that mycelia 1, 2, 3, 4, and 6 are alike in behaviour and 5 is different. Yet, in culture, mycelium 2 produces a very dense mat, mycelia 5 and 6 a moderately dense one, and 1, 3, and 4 a thin spreading one. Hence, no diagnostic macroscopic or microscopic differences in cultural characters have been discovered, and it has been found impossible to distinguish between the two groups unless the actual pairings are made.

The microscopic features of the mycelium of *Fomes pinicola* are described by Dr. Fritz as follows (21, p. 224): "Initial growth consisted of a very delicate type of irregularly branched, colourless hyphæ with frequent septa and clamp-connections. These were very fine, 1-2 μ or occasionally 4 μ wide, had very delicate walls and rather coarse, granular contents. In the submerged mycelium the same type of hypha appeared; but they were, on the whole, broader and more vigorous in appearance; 2-6μ with the majority 4 μ wide. On potato-

dextrose before the end of the first week there developed thick-walled, fibre-like threads with narrow lumina. These were colourless, very sparingly branched, uniform in width, unseptated, and with hyaline walls of uniform thickness. They also were fine, usually 2 μ but ranging from 1-4 μ in width. Both types were formed on all media except *corn*, on which the entire mat consisted of delicate thin-walled threads with septa and clamp-connections; but on *potato-dextrose* the fibre-like hyphæ developed earlier and were formed in greater profusion than on other media. Even in old cultures on *malt* the bulk of the aerial growth consisted of very fine threads of the first type. These delicate hyphæ were rendered more conspicuous on *Czapek's synthetic* agar owing to the development of somewhat thicker walls than in other mats, and the aerial growth on this medium was found powdered with a mass of fine crystals."

This description refers to mycelium isolated from sporophore tissue or decayed wood. Such a mycelium would be of *polysporous* origin. The types of hyphæ described, as well as chlamydospores, which develop in some cultures, are illustrated in plate II. Figures 12-15 show the thin-walled hyphæ with clamp-connections in the aerial mat, figures 17-18 a similar type from the submerged growth, figure 16 the type of thick-walled, fibre-like hypha, and figures 19-21 types of chlamydospores.

Mycelia of *monosporous* origin do not produce clamp-connections, otherwise they resemble those of polysporous origin in their microscopic features. They produce the thin-walled, irregularly branched hyphæ characteristic of the early aerial and the submerged growth (plate II, figs. 22-23), the thick-walled, sparingly branched fibre-like hyphæ (fig. 24), and chlamydospores (figs. 19-21). Clamp-connections, however, have never been found on a mycelium of monosporous origin, although some of them have been kept in culture for five years; i.e., so far none of the monosporous mycelia of *F. pinicola* has changed spontaneously from the haploid to the diploid state. Pairings of monosporous mycelia made immediately after the single spore cultures were isolated, and then duplicated three years later, gave identical results. In this *F. pinicola* differs from *Coprinus radians* (69a) and *C. Rostrupianus* (42), and the question of hetero-homothallism does not arise (69a). On the other hand, in the sexual stability of its monosporous mycelia *F. pinicola* resembles *Coprinus lagopus* (24a). After an extensive study of monosporous mycelia Hanna concluded that "this species may be considered as a strictly heterothallic species in which sexual mutations take place only occasionally. The existence of such a species does not accord with the hetero-homothallic theory of Vandendries."

With *Fomes pinicola* no difficulty has been experienced in obtaining pored surfaces and spores on diploid mycelia resulting from the pairing of two monosporous mycelia of opposite sex. Several times fruiting surfaces have developed on the malt agar plates and shed spores. Usually, however, tubes of prune agar were inoculated with the diploid mycelium and placed in the light at room temperature. Pored surfaces have developed, too, on paired cultures grown on Czapek's agar, but no spores were shed, although basidia, bearing sterigmata and spores, were found in the hymenium.

Monosporous mycelia grown under the same conditions as the compound mycelia usually show no signs whatever of fruiting. In three or four cases where monosporous mycelia have been grown in large tubes of prune agar for periods of three to four months thin areas have developed typical colour and a few shallow dædaloid pores (plate IX, fig. 1), but no spores have ever been shed and no basidia found on the pored surfaces. Evidently, then, diploid mycelia of *F. pinicola* fruit much more readily and normally than haploid mycelia from the same source.

D. SEXUAL STRAINS OR GEOGRAPHICAL RACES

Experiments made by Kniep with *Schizophyllum commune* (32), by Vandendries with *Panœolus campanulatus* (68) and *Coprinus radians* (69), by Brunswik with *Coprinus comatus, C. fimentarius, C. Friesii, C. lagopus, C. niveus,* and *C. picaceus* (9), and by Hanna with *C. lagopus* (24) have demonstrated the existence of different sexual strains which are perfectly cross fertile. Hanna used material collected from four widely separated points in Canada and from three places at Birmingham, England. Not only did he conclude that "complete fertility results when monosporous mycelia from wild fruit-bodies of different sexual strains are paired together," but that "English and Canadian strains of *C. lagopus* are similar morphologically and have been shown by the clamp-connection criterion to be identical."

A detailed analysis of the behaviour of monosporous mycelia of *Coprinus micaceus* (71, 71a) from widely separated spots in Canada and in Europe led Vandendries to the following interesting conclusions:—

(1) "Entre individus d'une même aire géographique, c'est la fertilité qui est normale." (2) "Entre souches éloignées, appartenant à deux aires géographiques distinctes, la stérilité est la règle. Ceci constitue un phénomène nouveau dans la biologie des Basidiomycètes." (3) "En Europe comme en Amérique, on trouve des cas de fertilité constituant des exceptions à la loi précédente." (4) "Il y a incompatibilité sexuelle entre races européennes et races canadiennes. . . ." Thus Hanna and Vandendries, each working with a species of *Coprinus,* arrive at different conclusions: when monosporous mycelia from different geographical areas are paired the former finds that complete fertility results, the latter that sterility is the rule, though there are exceptions. Perhaps Hanna's results might be considered comparable with those obtained by Vandendries with his Edmonton x Diekirch, and Minaki x Diekirch strains which were completely cross fertile, and in themselves constitute an exception as Vandendries noted. However, since complete fertility resulted in each of 694 pairings of monosporous mycelia of *C. lagopus* using material from three sources in Birmingham, England, and from six in Canada, as widely separated as Vancouver, B.C.; Edmonton, Alta.; Shellbrook, Sask.; Winnipeg, Man.; and Halifax, N.S., it seems more reasonable to expect that with this species complete fertility would be the rule rather than the exception, no matter what the source of the material. Only by the accumulation of a large amount of evidence obtained from experiments with many species of Basidiomycetes will it be possible to determine definitely which is the general mode of behaviour and which the exception. From this point of view the results of pairing monosporous mycelia of *F. pinicola* from the following localities may be of interest:—

(*a*) Cultures 283, 285A, 285B, 5770, 5778, from Ontario, Canada.

(*b*) Culture, 586, from France.

(*c*) Culture 928, from Sweden.

(*d*) Culture 562C, from British Columbia, Canada.

(*a*) and (*b*). *Material from Ontario and France.*—Five monosporous mycelia were taken at random from each of the following sources (see page 40) 283, 285A, 285B, 5770, 5778, and 586 and all possible pairings made with five monosporous mycelia from each of the other sources; that is twenty-five pairings each were made of 283 x 285A (five monosporous mycelia from 283 with five monosporous mycelia from 285A), of 283 x 285B, of 283 x 5770, of 285A x 586, etc. Since for two of these, 285A x 285B and 283 x 586, ten monosporous mycelia were selected from each source, requiring one hundred pairings in each

case, over four hundred pairings in all were made of these strains. Table X summarizes the results obtained in the pairings of 285A and 285B, and is typical of the results obtained throughout this series. As before the + sign indicates the presence of clamp-connections, the — sign their absence.

TABLE IX.—*Fomes pinicola.* All possible pairings of ten monosporous mycelia isolated from culture No. 285A. (Timagami, Ont.)

285A	1	2	4	6	7	8	3	5	9	10
1	—	—	—	—	—	—	+	+	+	+
2	—	—	—	—	—	—	+	+	+	+
4	—	—	—	—	—	—	+	+	+	+
6	—	—	—	—	—	—	+	+	+	+
7	—	—	—	—	—	—	+	+	+	+
8	—	—	—	—	—	—	+	+	+	+
3	+	+	+	+	+	+	—	—	—	—
5	+	+	+	+	+	+	—	—	—	—
9	+	+	+	+	+	+	—	—	—	—
10	+	+	+	+	+	+	—	—	—	—

TABLE X.—*Fomes pinicola.* All possible pairings of ten monosporous mycelia from culture No. 285B with ten monosporous mycelia from culture No. 285A.

285A	1	2	3	4	5	6	7	8	9	10
1	+	+	+	+	+	+	+	+	+	+
2	+	+	+	+	+	+	+	+	+	+
3	+	+	+	+	+	+	+	+	+	+
4	+	+	+	+	+	+	+	+	+	+
5	+	+	+	+	+	+	+	+	+	+
6	+	+	+	+	+	+	+	+	+	+
7	+	+	+	+	+	+	+	+	+	+
8	+	+	+	+	+	+	+	+	+	+
9	+	+	+	+	+	+	+	+	+	+
10	+	+	+	+	+	+	+	+	+	+

In every pairing but one (285A-1 x 586-2) clamp-connections were produced; that is each source belonged to a different sexual strain or geographical race, and these strains or races were all mutually fertile.

(*c*) *Material from Sweden.*—A year and a half later a culture of *Fomes pinicola (F. marginatus)* from *Betula* sp. was received from Stockholm, Sweden, through the kindness of Mr. E. J. Eliason. Monosporous mycelia were isolated and all possible pairings were made between five monosporous mycelia from this source and five from each of the sources in (*a*) and (*b*). Clamp-connections were formed in all but six of the three hundred pairings made; that is, in 98 per cent of the pairings, strains from sources (*a*) and (*b*) were completely cross fertile with those from (*c*), and table XI is typical of the results obtained.

TABLE XI.—*Fomes pinicola.* All possible pairings of five monosporous mycelia from culture No. 285B (Timagami, Ont.) with five monosporous mycelia from culture No. 928 (Sweden).

928	—	4	5	6	7	11
8	+	+	+	+	+	+
1	+	+	+	+	+	+
2	+	+	+	+	+	+
3	+	+	+	+	+	+
4	+	+	+	+	+	+

TABLE XII.—*Fomes pinicola.* All possible pairings of five monosporous mycelia from culture No. 283 (Timagami, Ont.) with five monosporous mycelia from culture No. 928 (Sweden)

928	1	9	11	15	16
6	+	+	—	+	+
7	+	+	—	+	+
8	+	+	+	+	+
1	+	+	—	+	+
2	+	+	—	+	+

Four of the six exceptions included mycelium 283-11 (see table XII) and two, mycelium 5770-1. Since these two monosporous mycelia behaved as all the others used in the series of pairings (*a*) and (*b*), and refused to behave normally in all subsequent series, it seems probable that their behaviour was due to loss of vigour, and that the six exceptions may be disregarded on that account, and the strain from Stockholm considered completely cross fertile with those from Ontario and from France.

(*d*) *Material from British Columbia.*—In the autumn of 1925 cultures were made from a large number of sporophores of *Fomes pinicola* collected in British Columbia. All of these cultures, however, refused to produce sporophores and spores, despite every effort that was made. Vandendries (71a) found, in working with cultures of *Coprinus micaceus*, a similar variation in readiness to produce sporophores. It was not until two years later that one culture, 562C, eventually shed spores. Nineteen monosporous mycelia were isolated and all possible pairings made of ten of them. The results did not differ from those shown in table VIII, and *F. pinicola* from this source too was shown to be heterothallic and bisexual. Then five monosporous mycelia from 562C were paired with five monosporous mycelia from every other source in (*a*), (*b*), and (*c*). The results were entirely unexpected for, with seven exceptions, clamp-connections were *not* produced in any of the 175 pairings made, and table XIII is typical of the series.

TABLE XIII.—*Fomes pinicola.* All possible pairings of five monosporous mycelia from culture No. 5778 (York Mills, Ont.) with five monosporous mycelia from culture No. 562C (Vancouver, B.C.)

	5778				
562C	3	4	5	6	7
7	−	−	−	−	−
8	−	−	−	−	−
9	−	−	−	−	−
10	−	−	−	−	−
11	−	−	−	−	−

TABLE XIV.—*Fomes pinicola.* All possible pairings of five monosporous mycelia from culture No. 586 (France) with five monosporous mycelia from culture No. 562C (Vancouver, B.C.)

	586				
562C	3	5	7	8	9
16	−	−	+	−	+
17	−	−	−	−	+
18	−	−	+	−	+
19	−	−	−	−	+
1	−	−	−	−	+

The seven exceptions occurred in the 562C x 586 pairings and are shown in table XIV. Even in this case there were only two of the five mycelia of 586, i.e. 586-7 and 586-9, involved. Hence, in this series, where monosporous mycelia from British Columbia were paired with monosporous mycelia from Ontario, France, and Sweden, only four per cent of the pairings were fertile, and complete sterility is the rule, though there are a few exceptions.

(*e*) *Summary.*—All the pairings made with monosporous mycelia of *Fomes pinicola* from different sources are summarized in table XV and several interesting facts are revealed.

TABLE XV.—*Fomes pinicola*. SUMMARY OF PAIRINGS OF MONOSPOROUS MYCELIA FROM VARIOUS HOSTS AND VARIOUS LOCALITIES

Number of monosp. mycelia.	Culture No.	Host	Locality	Paired with	Number of monosp. mycelia.	Culture No.	Host	Locality	Number of pairings	Fertile (+)	Sterile (−)
5	283	*Picea mariana*	Timagami, Ont.	x	5	285A	*Picea mariana*	Timagami, Ont.	25	25	0
5	283	"	"	x	5	285B	"	"	25	25	0
5	283	"	"	x	5	5770	*Picea* sp.	Guelph, Ont.	25	25	0
5	283	"	"	x	5	5778	*Prunus serotina*	York Mills, Ont.	25	25	0
10	285A	"	"	x	10	285B	*Picea mariana*	Timagami, Ont.	100	100	0
5	285A	"	"	x	5	5770	*Picea* sp.	Guelph, Ont.	25	25	0
5	285A	"	"	x	5	5778	*Prunus serotina*	York Mills, Ont.	25	25	0
5	285B	"	"	x	5	5770	*Picea* sp.	Guelph, Ont.	25	25	0
5	285B	"	"	x	5	5778	*Prunus serotina*	York Mills, Ont.	25	25	0
5	5770	*Picea* sp.	Guelph, Ont.	x	5	5778	"	"	25	25	0
10	586	Host unknown	France	x	10	283	*Picea mariana*	Timagami, Ont.	100	100	0
5	586	"	"	x	5	285A	"	"	25	25	0
5	586	"	"	x	5	285B	"	"	25	25	0
5	586	"	"	x	5	5770	*Picea* sp.	Guelph, Ont.	25	25	0
5	586	"	"	x	5	5778	*Prunus serotina*	York Mills, Ont.	25	25	0
5	928	*Betula* sp.	Stockholm, Sweden	x	5	283	*Picea mariana*	Timagami, Ont.	25	21	4
5	928	"	"	x	5	285A	"	"	25	25	0
5	928	"	"	x	5	285B	"	"	25	25	0
5	928	"	"	x	5	5770	*Picea* sp.	Guelph, Ont.	25	23	2
5	928	"	"	x	5	5778	*Prunus serotina*	York Mills, Ont.	25	25	0
5	928	"	"	x	5	586	$	France	25	25	0
								Total	675	669	6
5	562C	*Tsuga heterophylla*	Vancouver, B.C.	x	5	283	*Picea mariana*	Timagami, Ont.	25	0	25
5	562C	"	"	x	5	285A	"	"	25	0	25
5	562C	"	"	x	5	285B	"	"	25	0	25
5	562C	"	"	x	5	5770	*Picea* sp.	Guelph, Ont.	25	0	25
5	562C	"	"	x	5	5778	*Prunus serotina*	York Mills, Ont.	25	0	25
5	562C	"	"	x	5	586		France	25	7	18
5	562C	"	"	x	5	928	*Betula* sp.	Stockholm, Sweden	25	0	25
								Total	175	7	168

1. Clamp-connections were produced in each of the 325 pairings using material from deciduous and coniferous trees from various localities in Ontario. That is material from each source belonged to a different so-called sexual strain or geographical race and these strains or races were all mutually fertile.

The particular case of pairings between 285A and 285B is rather interesting. Any monosporous mycelium from sporophore 285A forms clamp-connections when paired with any monosporous mycelium of 285B, table X, showing that they belong to two different sexual strains. Yet sporophores 285A and 285B were growing at the same time on a black spruce. In working with *Coprinus lagopus* Hanna found (24, p. 442) that in pairings made of monosporous mycelia from two fruit-bodies arising from the same compound mycelium the mycelia " react together in every respect as though they had been isolated from a single fruit-body ". Apparently then there was more than one source of infection in this tree and the mycelium which produced 285A was of different origin from that which produced 285B. This theory is supported by the fact that a black line is produced when cultures 285A and 285B are grown in the same Petri plate culture. No such line is formed when the two inocula come from the same source, and frequently none develops when the two inocula come from spores and the tissue of the sporophore, or from the decayed wood on which the sporophore developed (see section XIII B). This result emphasizes the care which is necessary in studying, for instance, the progress of decay in individual trees.

2. In 350 pairings of European and Canadian strains of *Fomes pinicola, omitting strain 562C,* only six of the number failed to produce clamp-connections; i.e. the European and Canadian strains used were mutually fertile and, by the clamp-connection criterion, have been shown to be identical.*

3. Culture 586 from France was labelled *Fomes pinicola,* var. *marginatus,* and culture 928 from *Betula* sp. was submitted by E. J. Eliason, Stockholm, as typical of the *F. marginatus* form. With the exception of culture 562C, all pairings of the *Fomes pinicola* and *F. marginatus* forms were fertile so that, again, by the clamp-connection criterion, these two forms have been shown to be identical.

4. In pairings of monosporous mycelia of culture 562C from British Columbia with those from all other sources in Canada, Sweden, and France, 94 per cent of them remained sterile. This strain of *F. pinicola* duplicates in part results obtained by Vandendries with *Coprinus micaceus.* He found that sterility was the rule when monosporous mycelia from material from different geographical areas were paired; " . . . toute souche est fertile pour tous les individus de sa région, de même qu'elle est stérile pour tous les individus des regions éloignées ". However, experiments made so far with strains of *F. pinicola* from various regions indicate that, with this fungus, fertility is the rule. and sterility the exception.

*Recently monosporous mycelia have been obtained from culture 694, isolated from a sporophore of *Fomes pinicola* which grew on *Pinus* sp., York county, New Brunswick. These monosporous mycelia were paired with monosporous mycelia from each of the other sources in both Canada and Europe. Clamp-connections were formed in every pairing ***except those with culture 562C*** from British Columbia.

XIV. DESTRUCTION OF WOOD

Fomes pinicola causes a rot of sapwood and heartwood of coniferous and deciduous trees, but is particularly destructive to the former. It probably does most damage in stands of trees which have been killed by other agencies. Under such conditions it works with remarkable rapidity and soon renders the timber worthless.

The rot which it causes is quite characteristic, and readily recognizable, especially in the more advanced stages (plate I). At first it is characterized by a darkening of the wood, due to the removal of cellulose by the fungus. Even at this stage it is impossible to get a smooth surface with a plane, for the wood becomes brittle and breaks away irregularly. The removal of cellulose results in shrinkage of the wood causing the formation of longitudinal and horizontal cracks which become more and more numerous. Gradually these spaces are filled with wefts of white mycelium and in the final stages the wood is reduced to a mass of wood with charcoal-like fracture which absorbs water readily, but it is frequently dry and brittle, and always very friable. This mass is held together by the mycelial felts, which consist of closely intertwined fungous hyphæ and are similar to the mats formed on agar cultures. Badly decayed wood of this kind gives the characteristic red colour with phloroglucin and hydrochloric acid, but remains unchanged in chlor-zinc iodine, indicating the absence of cellulose in decayed wood.

Sections of the wood of *Abies* and *Picea,* which had been rotted by *Fomes pinicola,* were cut and examined. Evidence of the shrinkage which had occurred was common in most sections, particularly in the form of radial cracks in transverse sections. Vertical shrinkage seemed to result frequently in the separation of the cells of medullary rays causing lengthwise cracks in the rays as seen in longitudinal radial sections.

Hyphæ were frequent both in the tracheids and medullary ray cells. They were all hyaline and thin-walled, but of two distinct types (1) the usual branched hyphæ, with clamp-connections, varying from 6-7 μ to 2-3 μ in width, but commonly 2-3 μ; and (2) hyphæ which were exceedingly fine, sparsely septate, and very much branched. They developed so profusely that they completely filled the lumen of the tracheids.

The work of the fungus in medullary ray cells is evident not only from the presence of hyphæ which grow in every direction through the rays but also from the presence of bore holes and a great number of enlarged pits. Hyphæ were found frequently passing through these enlarged pits and entering the adjoining tracheid. Not uncommonly the cells of the medullary rays were filled with a deeply staining granular substance.

Similarly, in the tracheids, bore-holes made by the fungus and enlarged pits were very common. Frequently longitudinal slits occurred in the cell wall on both sides of bordered pits and bore-holes. Hyphæ passed from one tracheid to another in radial or tangential direction. They made use of the bordered pits, even of the ones in the tangential walls of the summer wood, but at the same time bore-holes were frequently found passing through the border of a bordered pit. The most characteristic feature, microscopically, both in longitudinal and transverse sections was the presence of rows and groups of cells in which the lumen was completely filled with a very densely woven mass of the fine, much-branched hyphæ, so closely packed that, in cases where the cell wall had broken away, the mycelium still retained the shape of the cell.

Sections of the wood of *Tsuga* which had been inoculated with *Fomes pinicola* and grown in culture showed similar evidences of the work of this fungus in the presence of bore-holes, hyphæ with clamp-connections, splitting of the cell wall, and the presence of tracheids completely filled with mycelium.

XV. SUMMARY

1. A list of 91 hosts is given for *Fomes pinicola* (Sw.) Cooke. [*Fomes marginatus* (Fr.), *Fomes ungulatus* (Schaeff.) Sacc.]

2. Spores of *F. pinicola* were sown in twenty-five different substrata and found to germinate in many of them. Germination took place at temperatures ranging from 8° to 35°C. Light retarded but did not inhibit germination.

3. Sporophores of *F. pinicola* have been obtained in cultures made on malt and on prune agar, and on Czapek's synthetic liquid medium containing various substitutes for the dextrose of the formula; and on eleven varieties of coniferous and deciduous wood, with rudimentary sporophores on fourteen others. The context of the sporophores of *F. pinicola* produced in culture was typical both in colour and texture, and the spores produced were viable.

4. A *Fomes*-type of fruit-body with three definite pore-layers, each of which shed spores, developed in a culture on wood of *Pinus divaricata*.

5. The mycelium of *F. pinicola* grew at temperatures ranging from 8° to 35°C. with an optimum temperature of about 27° to 29°C. Growth was retarded at both the maximum and minimum temperatures. Sporophore production was inhibited at 8°C. The mycelium grew best in an acid medium, preferably one with a pH value of 4·8 to 5·2.

6. Young cultures of mycelia from various sources differ from one another in rate of growth, colour production, and texture of the mycelial mat; older cultures tend to become more uniformly white and felted. These differences have been ascribed to individual variation rather than to host influence. Despite these variations a study of the macroscopic and microscopic characters of the mycelium made it possible to identify this fungus with a fair degree of accuracy.

7. A study of mixed cultures of *F. pinicola* gave no definite clue as to the cause of the frequent formation of a line of demarcation or a space of aversion between two mycelia from different sources.

8. A method of isolating monosporous mycelia of *F. pinicola* or of similar forms is described.

9. *F. pinicola* is a heterothallic bisexual species.

10. Many so-called sexual strains, or geographical races, which were completely cross fertile, were found.

11. Monosporous mycelia of *F. pinicola* which have been kept in culture for five years, have remained in the haploid condition. In this they are unlike monosporous mycelia of two heterothallic bisexual species of *Coprinus, C. Rostrupianus* investigated by Miss Newton and *C. radians* investigated by Vandendries, many of which were found to change spontaneously from the haploid to the diploid condition.

12. Monosporous mycelia of *F. pinicola* isolated from sporophores from deciduous hosts were mutually fertile with those from sporophores from coniferous hosts; monosporous mycelia from cultures from France and Sweden were mutually fertile with monosporous cultures from all Canadian strains except that from British Columbia. These results, interpreted in the light of the clamp-connection criterion for the identity of species, furnish experimental evidence in support of the generally accepted conclusions (1) that *F. marginatus* and *F. pinicola* are one and the same species, and (2) that the European and American forms of this fungus are identical.

13. Monosporous mycelia of a strain of *F. pinicola* from British Columbia remained completely sterile when paired with monosporous mycelia from any other Canadian strain or from the strain from Sweden, and were only partially fertile with those from a strain from France. These results duplicate, in part, those obtained by Vandendries with *Coprinus micaceus*.

14. The macroscopic and microscopic characters of rot caused by *F. pinicola* are described. Typical rot was obtained in artificial culture.

This investigation was begun at the Department of Botany, University of Toronto, during the tenure of a studentship and fellowship granted by the National Research Council, and completed at the Division of Botany, Experimental Farm, Ottawa. I wish to express my gratitude to Professor J. H. Faull for his kindness in supplying a great part of the material used, and especially for his valuable advice and criticism throughout the progress of the work; to Prof. A. H. R. Buller, who suggested the problem; and to Mr. H. T. Güssow, Dominion Botanist, for his unfailing interest in the investigation.

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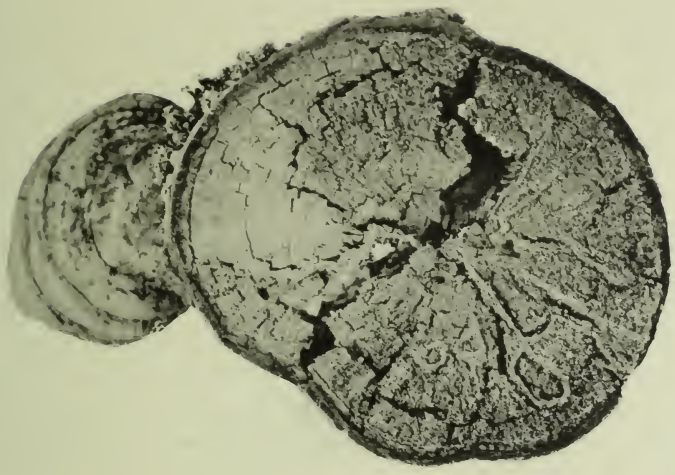
XVII. DESCRIPTION OF PLATES

PLATE I

Fig. 1 (upper).—Transverse section of block of *Tsuga heterophylla* showing ungulate sporophore of *Fomes pinicola* and advanced stage of rot. $\frac{3}{8}$ nat. size.

Fig. 2 (lower).—Longitudinal section of block of *Tsuga heterophylla* showing applanate sporophore and stages of rot. $\frac{1}{4}$ nat. size.

Plate I



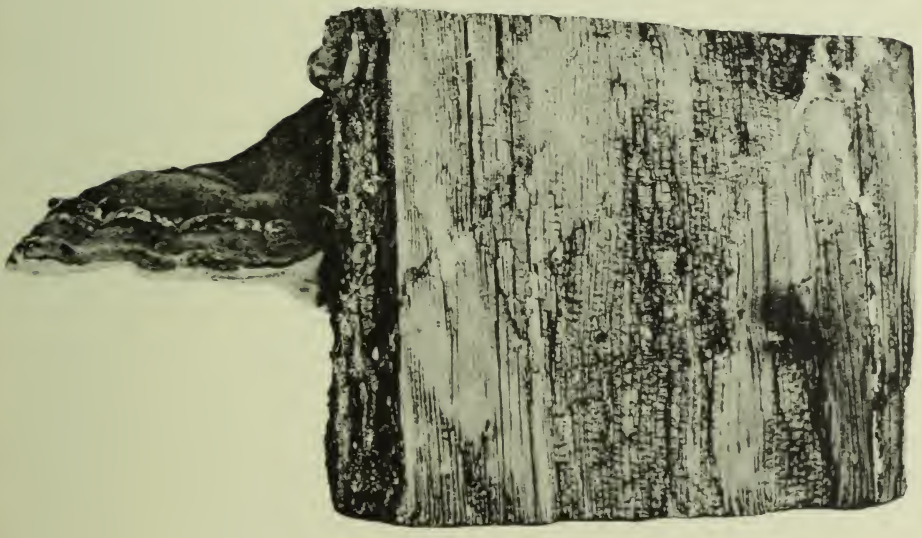


Plate II

Fig. 1.—Spores of *Fomes pinicola*. x 800.

Figs. 2-5.—Germinating spores of *F. pinicola*. x 490. In 1% lactose.

Figs. 6, 7.—Germinating spores of *F. pinicola*. x 490. In prune agar 3 days.

Fig. 8.—Germinating spore of *F. pinicola*. x 490. Same spore as fig. 6, 24 hours later.

Fig. 9.—Germinating spore of *F. pinicola*. x 490. In beet agar 2 days.

Fig. 10.—Germinating spore of *F. pinicola*. x 490. In prune agar 3 days.

Fig. 11.—Germinating spore of *F. pinicola*. x 490. In parsnip agar 2 days.

Figs. 12-14.—Types of thin-walled hyphæ with clamp-connections. From aerial mycelium of tissue culture on prune agar. x 800.

Fig. 15.—As figs. 12-14. x 490.

Fig 16.—Thick-walled fibre-like hypha from aerial mycelium of same culture as fig. 12. x 800.

Figs. 17-18.—Types of thin-walled, irregularly branching hyphæ from submerged mycelium of a tissue culture on prune agar. x 490.

Figs. 19-21.—Chlamydospores from submerged mycelium of a month-old tissue culture on malt agar. x 800.

Figs. 22-23.—Types of thin-walled hyphæ from submerged growth of a monosporous mycelium on malt agar. x 800.

Fig. 24.—Fibre-like hypha from submerged growth of a monosporous mycelium on prune agar. x 800.

Fig. 25.—Thin-walled hypha with clamp-connections obtained by pairing two monosporous mycelia, 158-2 x 5770-2, on Czapek's synthetic agar. x 800.

Fig. 26.—Hypha with yellow-brown coarsely granular contents from black line area in a paired culture of 283 x 286A. x 800.

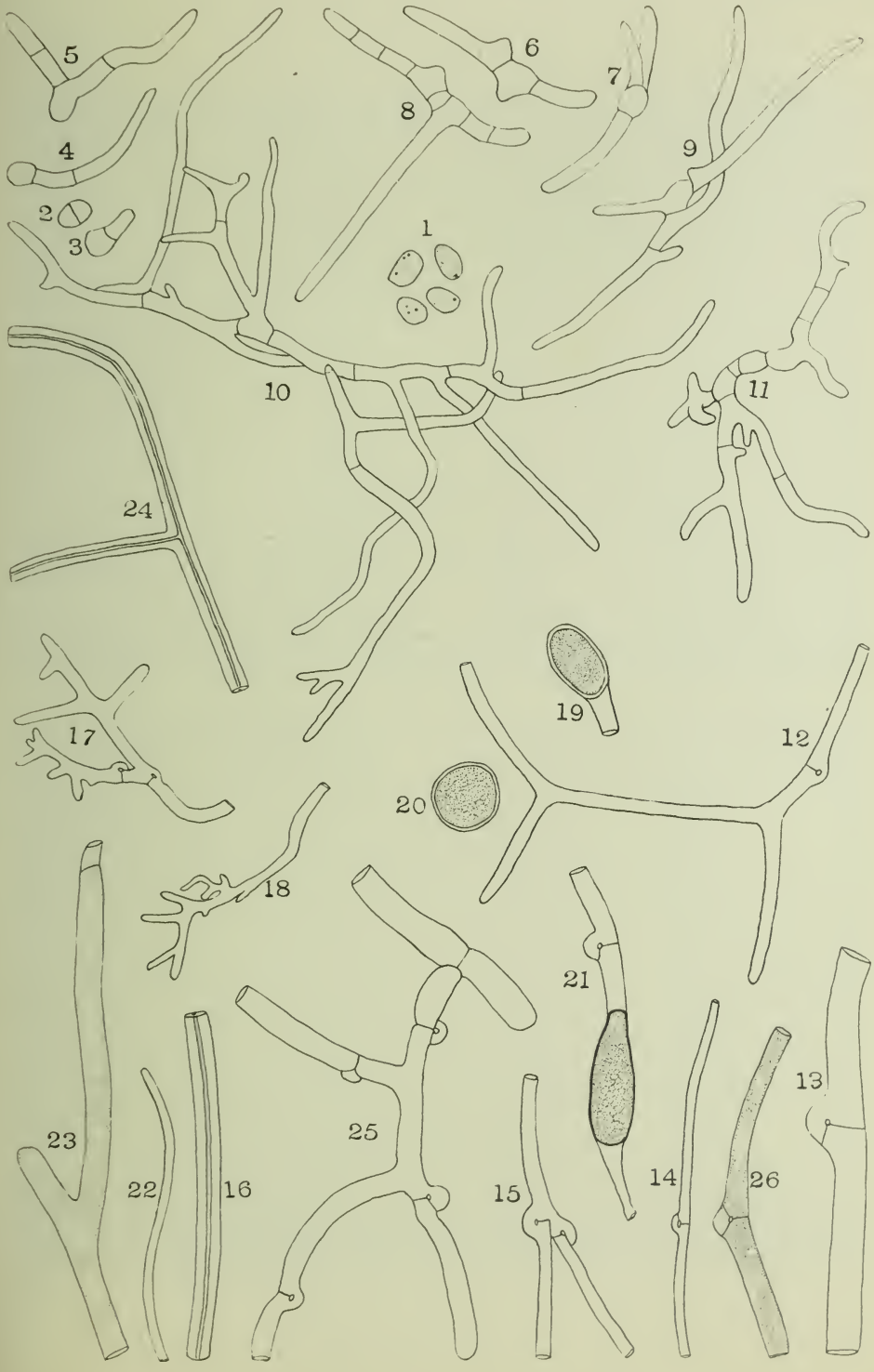

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Plate III

Cultures of *Fomes pinicola* grown in diffuse light at room temperature. Nat. size.

Figs. 1-3.—Culture 158, showing felted mycelium, sporophores, and spore-deposits (masking pores in figs. 2 and 3).

Fig. 4.—Culture 219. Sporophore with coarse pores. Indication of spore-deposit on right-hand side of tube.

Fig. 5.—Culture 158. Sporophore and typical growth on Czapek's synthetic agar.

Fig. 6.—Culture 5770 from *Picea mariana* showing pores and spore-deposit.

Fig. 7.—Culture 5775 from *Abies balsamea* showing pores and spore-deposit.

Fig. 8.—Culture 5657 from *Betula alba* showing pores and spore-deposit.

Fig. 9.—Culture 5778 from *Prunus serotina* showing pores and spore-deposit.

Fig. 10.—Culture 5769 from *Pinus Strobus* showing pores and spore-deposit.

Figs. 6-10.—The similarity of texture of mycelial mat in old cultures is shown.

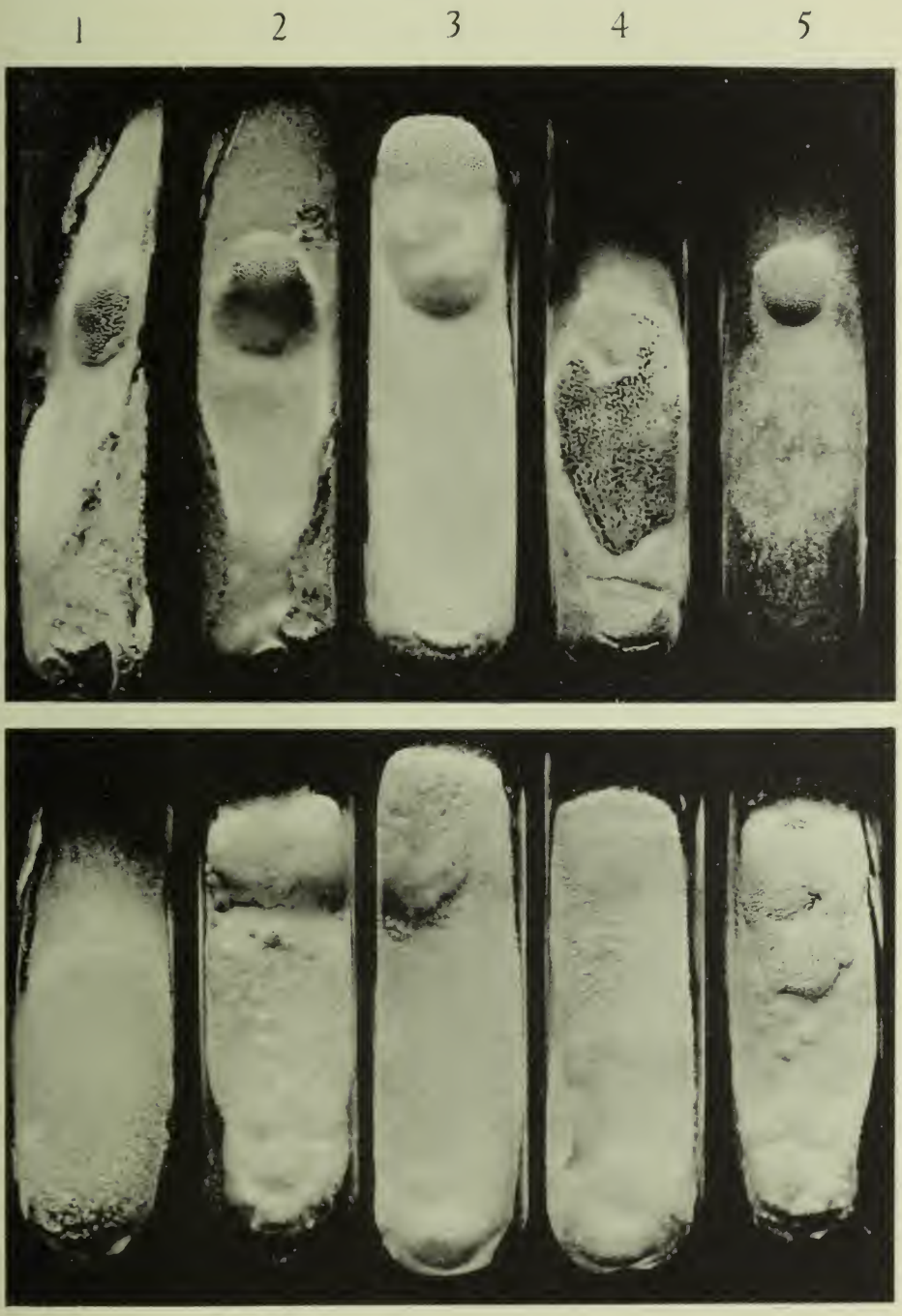
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Plate IV

Figs. 1-4.—*Fomes pinicola.* Ten-day old cultures of 158 on prune agar. Nat. size.
Fig. 1.—Incubated at 35°C.
Fig. 2.—Incubated at 31°C.
Fig. 3.—Incubated at 29°C.
Fig. 4.—Incubated at 22°C.
Figs. 5-8.—Cultures of *F. pinicola* from *Larix.* On prune agar.
Fig. 5.—Culture 5772.
Figs. 6-8.—Culture 160.

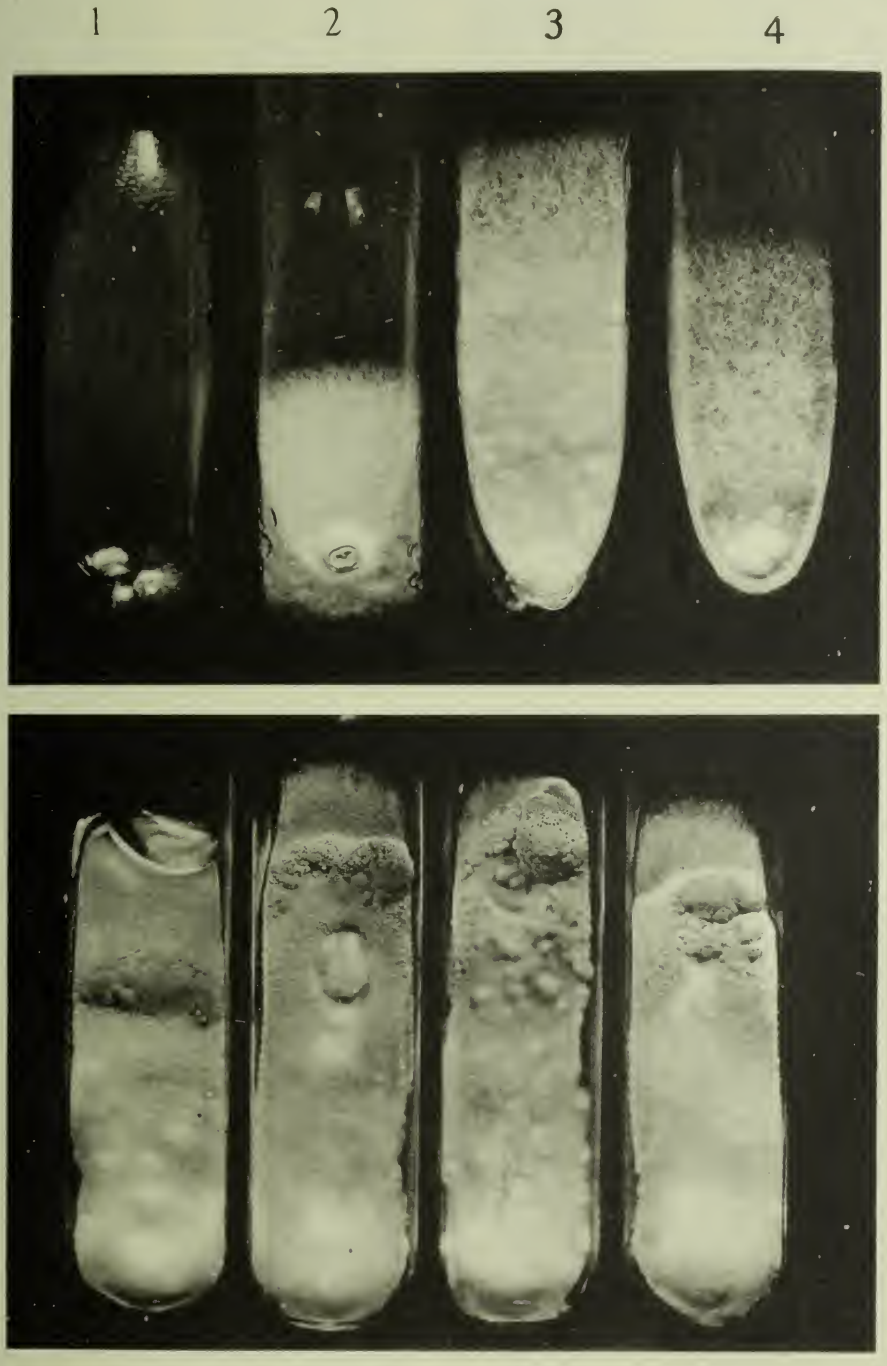
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Plate V

Fomes pinicola culture 219 on wood blocks. Nat. size.

Fig. 1.—On *Pinus divaricata*. Sporophore rudiment, 4 months after inoculation.

Fig. 2.—On *Pinus divaricata*. Pores and spores of first hymenial layer, 6 months after inoculation.

Fig. 3.—On *Pinus divaricata*. Pores and spores of second hymenial layer, 8 months after inoculation.

Fig. 4.—On *Pinus divaricata*. Pores and spores of third hymenial layer, 10 months after inoculation.

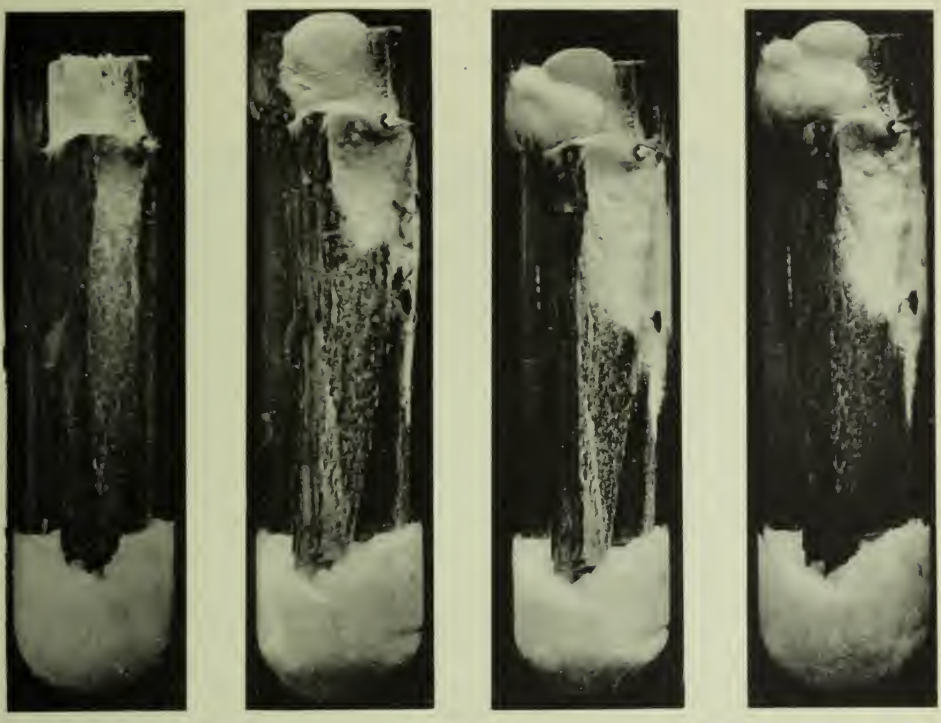
Fig. 5.—On *Tilia*. Mycelium and pores, 20 months after inoculation.

Fig. 6.—On *Ulmus racemosa*. Dense mycelium, 20 months after inoculation.

Fig. 7.—On *Populus tremuloides*. Sporophore, scant mycelium, 20 months after inoculation.

Fig. 8.—On *Carya ovata*. Sporophore, dense mycelium, 20 months after inoculation.

Fig. 9.—On *Acer saccharum*. Sporophore, dense mycelium, 20 months after inoculation.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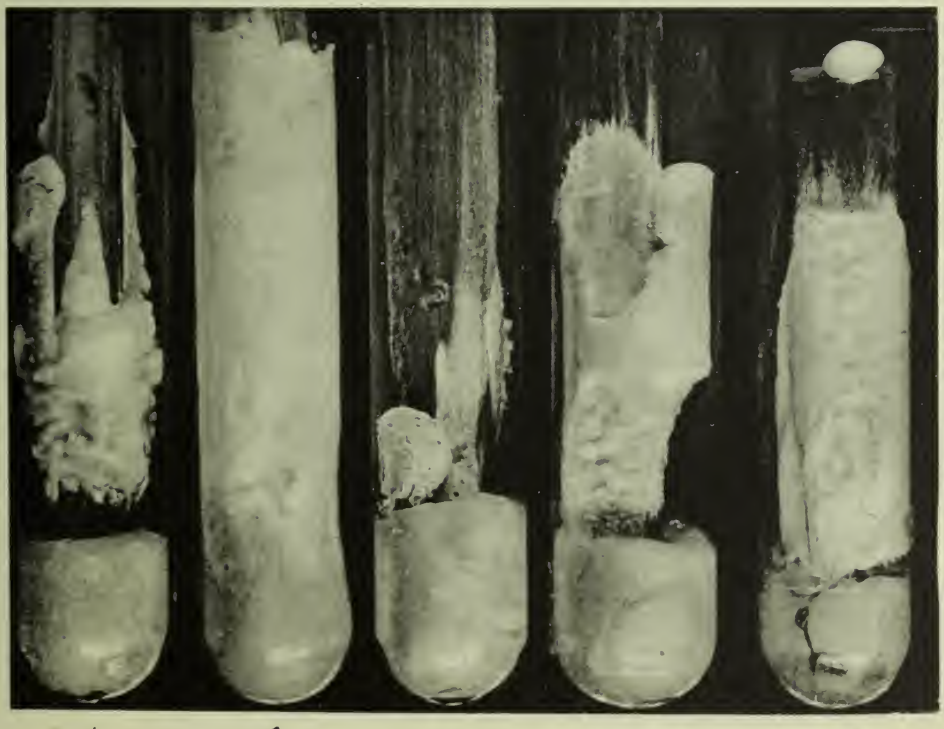
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Plate VI

Ten-day old paired cultures of *Fomes pinicola* on prune agar. ¾ nat. size.

Fig. 1.—Culture 225 paired with culture 219. Space between mycelia.

Fig. 2.—Culture 5776 paired with culture 5775. Space between mycelia.

Fig. 3.—Culture 5769 paired with culture 5775. Complete fusion.

Fig. 4.—Culture 5772 paired with culture 160. Complete fusion.

Fig. 5.—Culture 5770 paired with culture 219. Line of demarcation.

Fig. 6.—Culture 5770 paired with culture 225. Line of demarcation.

Plate VI

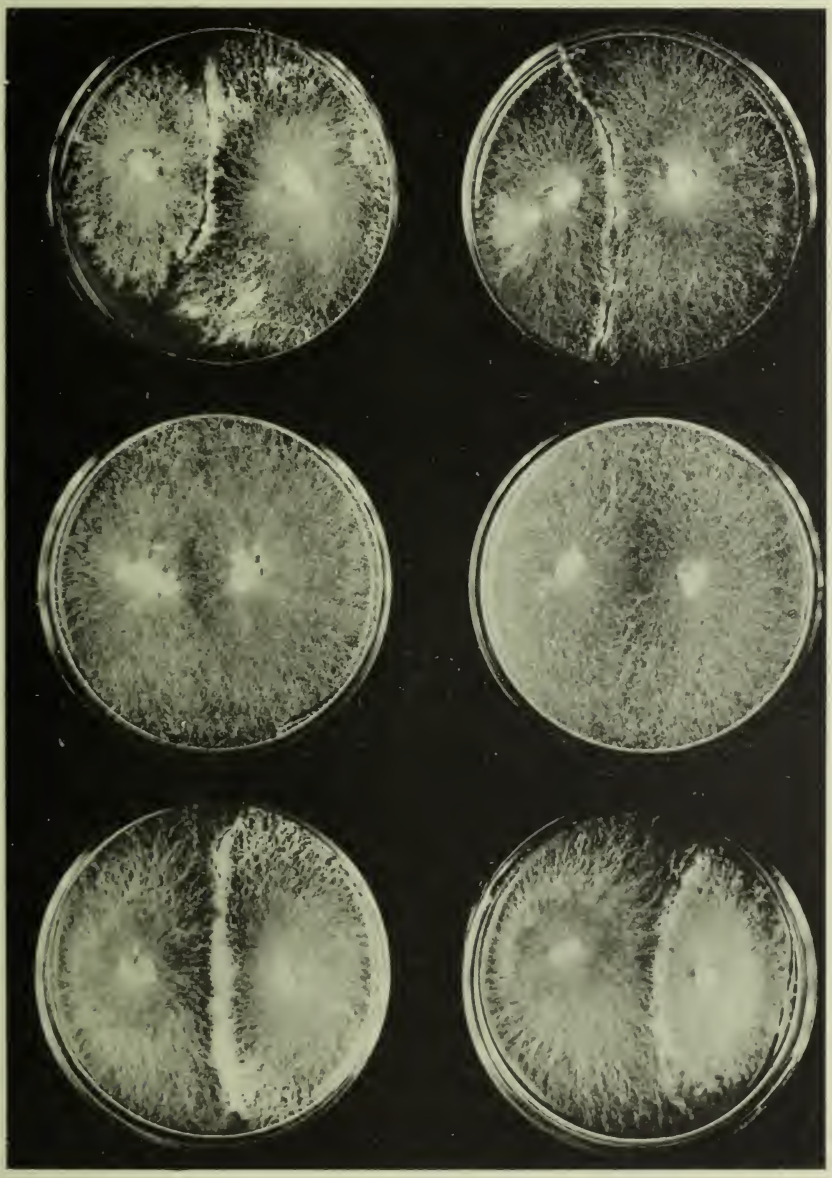

Plate VII

Ten-day old paired cultures of *Fomes pinicola* on prune agar. ⅔ nat. size.

Fig. 1.—Culture 5769 paired with culture 219. Line of demarcation, drops.

Fig. 2.—Culture 5657 paired with culture 158. Line of demarcation.

Fig. 3.—Culture 5775 paired with culture 5657. Space.

Fig. 4.—Culture 160 paired with culture 158. Space.

Fig. 5.—Culture 5657 paired with culture 219. Line of demarcation.

Fig. 6.—Culture 219 paired with culture 160. Line of demarcation.

Plate VII

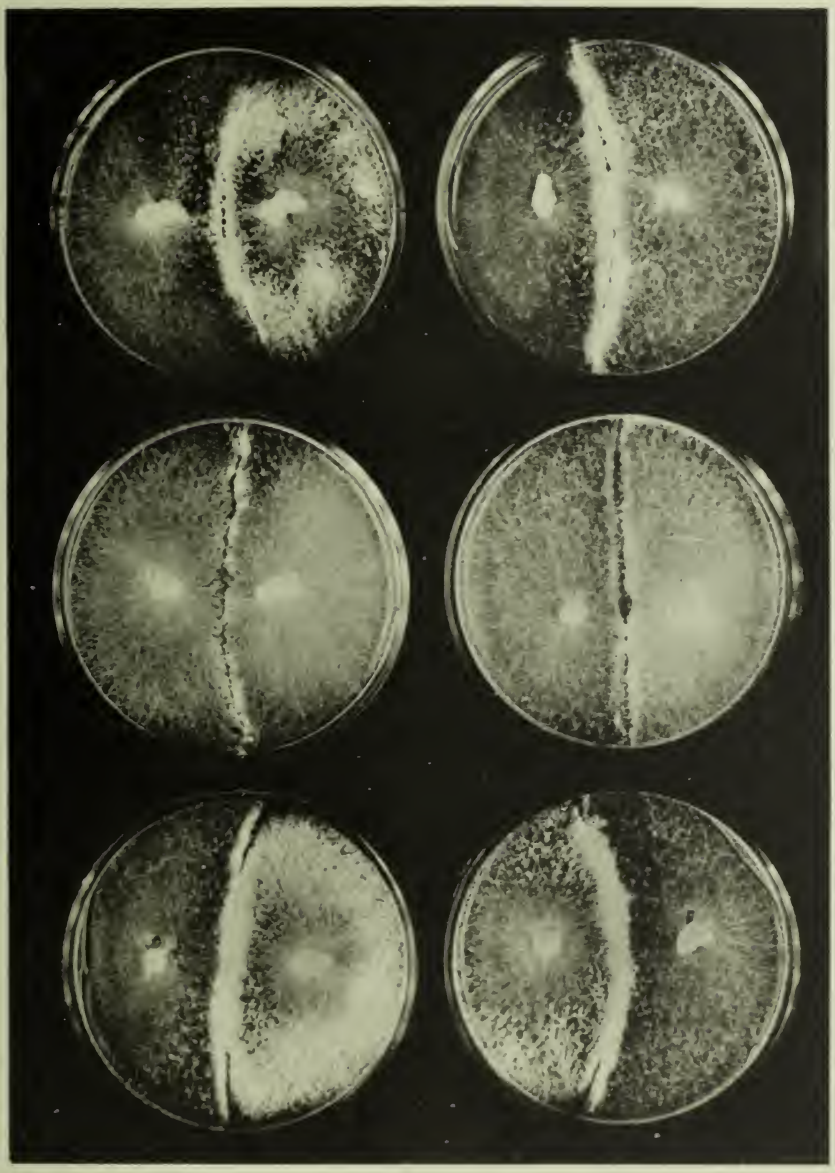

Plate VIII

Cultures of *Fomes pinicola*. Figs. 1-4 showing similarity, figs. 5-8 showing variations in mycelial mat. Nat. size.

Fig. 1.—574C, mycelium from *Tsuga heterophylla*, Vancouver, B.C.

Fig. 2.—694, mycelium from *Pinus* sp., New Brunswick.

Fig. 3.—511, mycelium from *Tsuga heterophylla*, Queen Charlotte city, B.C.

Fig. 4.—300, mycelium from *Picea canadensis*, Timagami, Ont.

Fig. 5.—304, mycelium from *Populus grandidentata*, Timagami, Ont.

Fig. 6.—306, mycelium from *Pinus Strobus*, Timagami, Ont.

Fig. 7.—694, mycelium from *Pinus* sp., New Brunswick.

Fig. 8.—559, mycelium from *Tsuga heterophylla*, Vancouver, B.C.

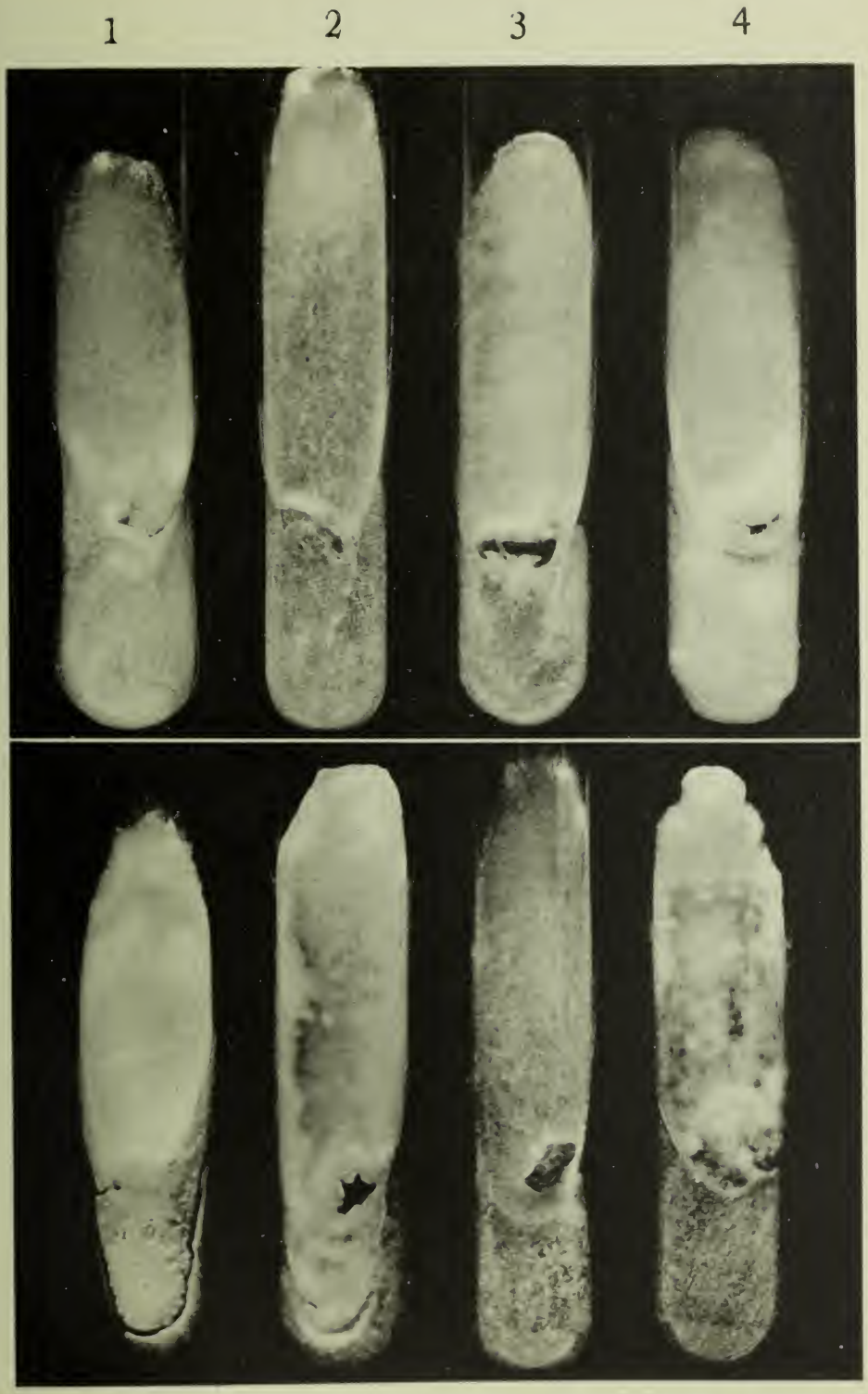
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Plate IX

Fig. 1.—*Fomes pinicola.* 4 months' old culture of monosporous mycelium 158-2 showing attempt at sporophore formation. Natural size.

Fig. 2.—Tissue culture 158 (left) paired with a polysporous mycelium obtained from germination of spores shed by 158. Mycelia do not fuse. Slightly reduced.

Fig. 3.—Tissue culture 158 (upper and larger mycelium) paired with two monosporous mycelia derived from it. A line of demarcation is formed where each monosporous (haploid) mycelium meets the polysporous (diploid) mycelium. A space remains without aerial mycelium where the two monosporous mycelia meet. Slightly reduced.

Plate IX

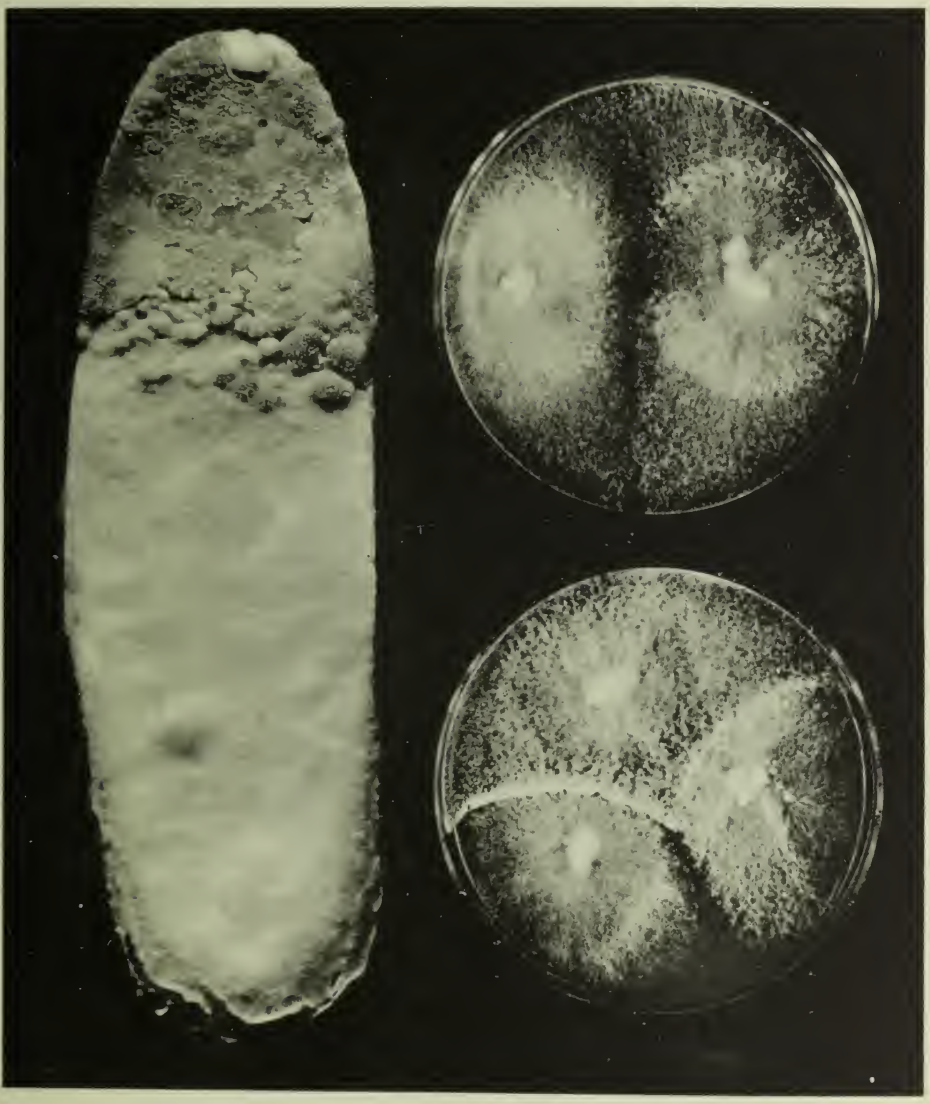
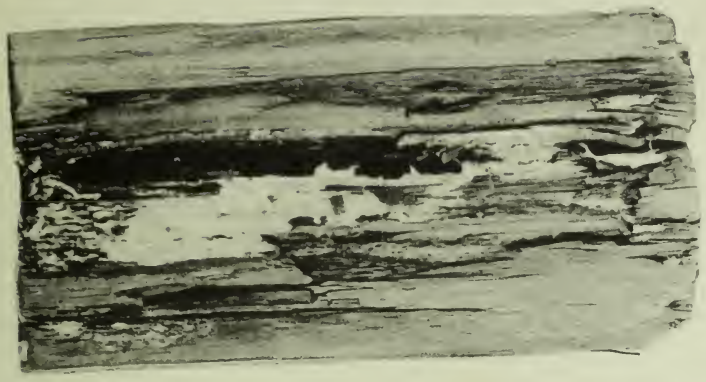


PLATE X

Typical rot and sporophore rudiments obtained by artificial inoculation of blocks of *Tsuga heterophylla* with *Fomes pinicola*.



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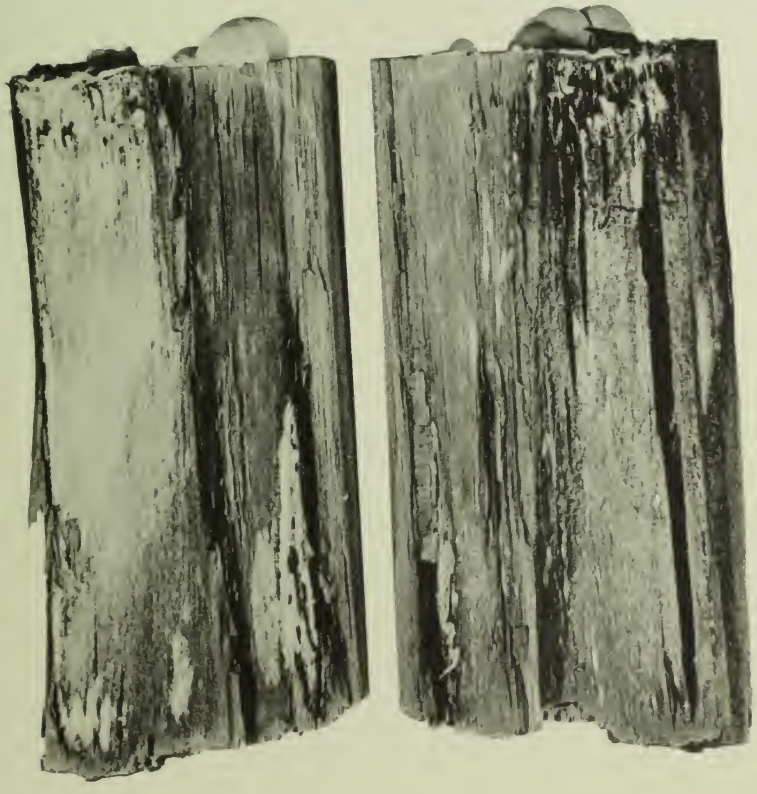

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STUDIES IN FOREST PATHOLOGY

I. Decay in Balsam Fir (*Abies balsamea*, Mill.): by A. W. McCallum, Forest Pathologist.

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